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THE LIFE HISTORY OF *PROTEOCEPHALUS TUMIDOCOLLUS* WAGNER, 1953, (CESTODA), IN RAINBOW TROUT[1]

EDWARD D. WAGNER
School of Tropical and Preventive Medicine
College of Medical Evangelists, Loma Linda, California

INTRODUCTION

This paper reports the results of studies made on the life history of *Proteocephalus tumidocollus* Wagner (1953), and on the effects of temperature on the development of the tapeworm larvae.

The history of proteocephalan life cycle studies begins with Gruber (1878) who was the first to discover a larval cestode in the body cavity of a cyclops. Meggitt (1914) made the first complete study of a fish cestode life cycle when he traced the development of *Proteocephalus filicollis* by experimental methods. The onchosphere in the egg was ingested by *Cyclops varius* and developed into a procercoid larva. The cycle was completed when the infected cyclops were eaten by stickleback fish.

The developmental cycle of *Proteocephalus torulosus* was experimentally studied by Wagner (1917). *Cyclops strenuus* and *Diaptomus castor* were found to be first intermediate hosts and a carp, *Cyprinus orfus,* the definitive host. Wagner studied in detail the morphology of the procercoid larvae in the intermediate hosts.

Cooper (1915), Bangham (1925, 1927), Hunter (1928), and Hunter and Hunter (1929) made contributions to the life cycle of *Proteocephalus ambloplitis,* a worm parasitic in the black bass, *Micropterus dolomieu.* Intermediate hosts were shown to be species of *Eucyclops, Macrocyclops,* and *Cyclops.*

Life history studies were made by Essex (1928) on *Corallobothrium fimbriatum* parasitic in *Ictalurus, Leptops,* and *Ameiurus.* Hunter (1929) made similar studies on *Proteocephalus pinguis* parasitic in pickerel (*Esox*). Preliminary studies on the life cycle of *P. primaverus* were made by Neiland (1952) who experimentally infected *Diaptomus franciscanus.*

METHODS AND MATERIALS

Life history studies were conducted in outdoor laboratories at Loma Linda, California. Trout eggs were provided by the California Trout Company, Mentone. All fish used experimentally were hatched in troughs, and experiments involving the fish were conducted in these containers. The redwood troughs used were 1 foot wide by 5 feet long and 1 foot deep, and covered by a metal screen. Inflowing well water was cooled to about 14° C. with a temprite photographic-type water

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cooler. The water temperature in the troughs was then maintained at approximately 12–18° C. The water was filtered through charcoal and gravel to remove chlorine and foreign matter that might be present. Mortality due to parasitic or bacterial diseases did not occur and all control fish gave negative results for parasites.

The young fish were maintained on finely ground frozen beef liver, while older fish received a mixture of ground beef liver and lung, and horse meat.

The cyclops used in these experiments were raised in the laboratory, or they were taken from an isolated tank. None of the several hundred cyclops examined from the tank was infected with helminth parasites, nor did an infection result within 10 weeks when several thousand of them were fed to 13 control fish. The copepods flourished when maintained in the laboratory at 10–20° C. and fed sparingly on live yeast and tropical fish food.

When worms, recently removed from the trout, were placed in water numerous eggs were expelled. These eggs were used in experimentally infecting cyclops.

In examining cyclops and other organisms for parasitic infection these animals were held in place on a glass slide by the pressure of a 12 mm. square cover glass. The cover glass could be moved so as to roll the organism into a more favorable position for observation under the microscope. The parasites were more readily detected when the host was viewed from its dorsal aspect.

Thermostatically controlled rooms and refrigerators were employed in certain of these experiments.

Infections in the definitive host were accomplished by placing young fish (1 to 1.5 inches in length) in 1-, 3-, or 5-gallon aquaria depending on the number of fish. The larger containers were provided with a water exchange system in which cooled water from one of the troughs was siphoned in, and in which the overflow tube was fitted with woven nylon to prevent the escape of cyclops. The fish and intermediate hosts were maintained together until no more cyclops could be seen in the water, after which time the contents of that aquarium was poured into a coarse sieve held over a plankton net. The sieve caught the fish while the net aided in recovering any cyclops present.

OBSERVATIONS

I. Intermediate Hosts

Negative results were obtained when large numbers of freshly expelled tapeworm eggs were mixed with fresh ground beef liver and fed to 16 fingerling trout for 2 days, indicating that direct infection of the definitive host did not occur.

Egg-viability tests showed that the eggs remain infective to cyclops for 13 days at room temperature, and retain their motility for more than a month at 0°, 1°, 5°, and 10° C., 19 days at 20° and 26° C., and 8 days at 32° C.

Of the various microcrustacea (Table I) collected from the hatchery reservoirs only species of *Cyclops, Eucyclops* and *Tropocyclops* were found to be naturally infected with developing tapeworm larvae.

When large numbers of tapeworm eggs were placed in a small container (60 mm. petri dish) with *Cyclops vernalis* it was observed that the cyclops ingested eggs as early as 5 minutes after the eggs were added to the dish. The escaped onchospheres could be seen in the foregut, and within 10 minutes after being ingested some larvae had arrived in the lower portion of the intestine. The first larvae had penetrated into the body sinuses within 25 minutes after being eaten.

The intensity of infection is probably governed by the degree of concentration of eggs. The number of larvae observed in naturally infected cyclops was 1 to 4, while as many as 30 were counted in some of the experimentally exposed cyclops. The largest number of fully developed larvae in cyclops was about 15. The intermediate hosts continued to ingest eggs for many days, or as long as viable eggs were present in the dish. Thus several days following the beginning of an experiment, various sizes of larvae were to be seen within a host.

During these studies it was observed that the infection rate in *Cyclops vernalis* was nearly 100 percent on many occasions when this species was used for experimental work. On 2 occasions, however, this species failed to become infected with tapeworm larvae. The first occasion resulted when a two-year old *Cyclops vernalis* culture was compared to a new culture for infections. No cyclops in the old culture were found to contain larvae, while those in the new culture of *C. vernalis* were heavily infected. Perhaps inbreeding in the two-year-old culture may have produced a physiological change which rendered the cyclops unsuitable as a host.

On the second occasion new cultures of *Cyclops vernalis* and *Tropocyclops prasinus* were placed in the same container and were exposed to tapeworm eggs. No *C. vernalis* were found infected with larvae, while the *T. prasinus* were heavily infected.

TABLE I.—*Microcrustacea examined for infections*

Species	Naturally infected	Experimentally infected
Cyclops vernalis Fisher 1853	+	+
Eucyclops agilis (Koch) 1838	+	+
Eucyclops speratus (Lilljeborg) 1901	+	+
Tropocyclops prasinus (Fisher) 1860	+	+
Macrocyclops albidus (Jurine) 1820	–	–
Paracyclops fimbriatus poppei (Rehberg) 1880	–	–
Cypridopsis vidua (O. F. Müller) 1776	–	–
Herpetocypris cheureuxi (Sars) 1896	–	–
Simocephalus vetulus (O. F. Müller) 1776	–	–
Eulimnadia sp.	–	–

Larval development: The following description is based upon the growth of the larvae at 20° C. which is about optimal.

The onchosphere usually escaped from the middle membrane shortly after the egg was expelled from the tapeworm. It showed increased motility when free between the middle and outer membrane. Movements consisted of slight changes in body shape. The hooklets were usually moved in unison, rather than individually. The onchosphere within freshly expelled eggs measures 0.024 to 0.027 mm.

Growth within the cyclops was gradual at first, the embryo having doubled its size in 5 days. After 20 days there was a marked leveling off in the growth rate. The maximum length attained by a larva when fully extended was 0.47 mm. Within a month after ingestion of eggs the cyclops and larvae died.

Calcareous bodies of various sizes and shapes increased in number within the growing larvae. These refractive granules were first seen after 9 days of development, and the number did not appear to be constant in the different larvae at any stage of development.

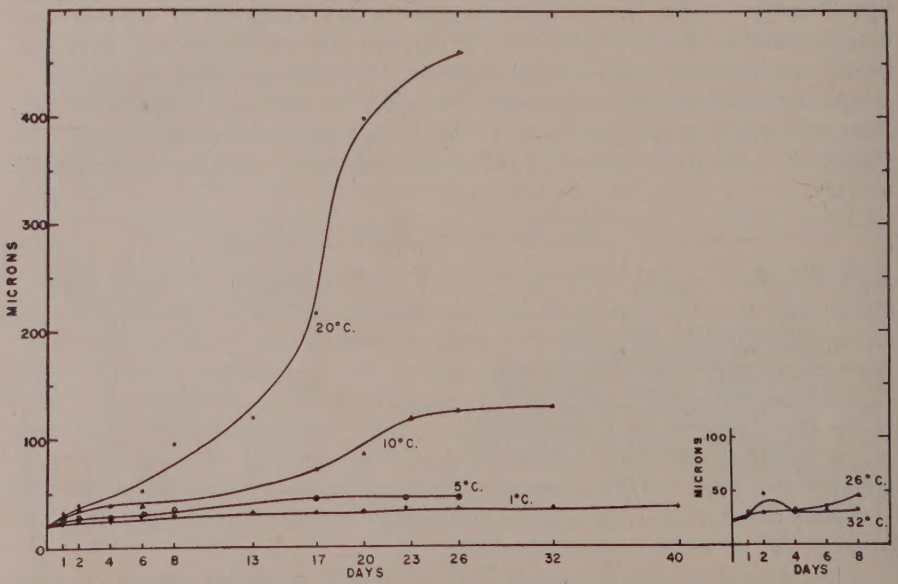
The first indication of sucker formation, including the fifth or apical sucker, was at 13 days. Three or 4 days later suckers appeared to be fully developed.

At 15 days a caudal appendage, the cercomer, was formed. The constriction between it and the body became gradually narrowed and the structure was shed

about 4 days later. The cercomer was motile while attached and for some time after being shed. Only rarely was a hooklet seen within it.

Temperature effects on development: An experiment was conducted to determine the effects of temperature on the growth and development of the procercoid larvae within the intermediate host. Freshly expelled tapeworm eggs were placed in 90 mm. petri dishes containing spring water. Approximately 50 cyclops were added to each container and the containers were placed in a refrigerator or in a room in which a constant temperature was maintained. Thus, one dish was kept at each of the following temperatures: 1°, 5°, 10°, 20°, 26°, and 32° C.

The rate of development is shown in Graphs I and II. The larvae were measured when in living cyclops without excess pressure from the cover glass. Measurements of the largest larvae only were recorded since the smaller forms probably



GRAPH I. Rate of growth of tapeworm larvae in cyclops at various temperatures. Growth is represented as increase in total length.

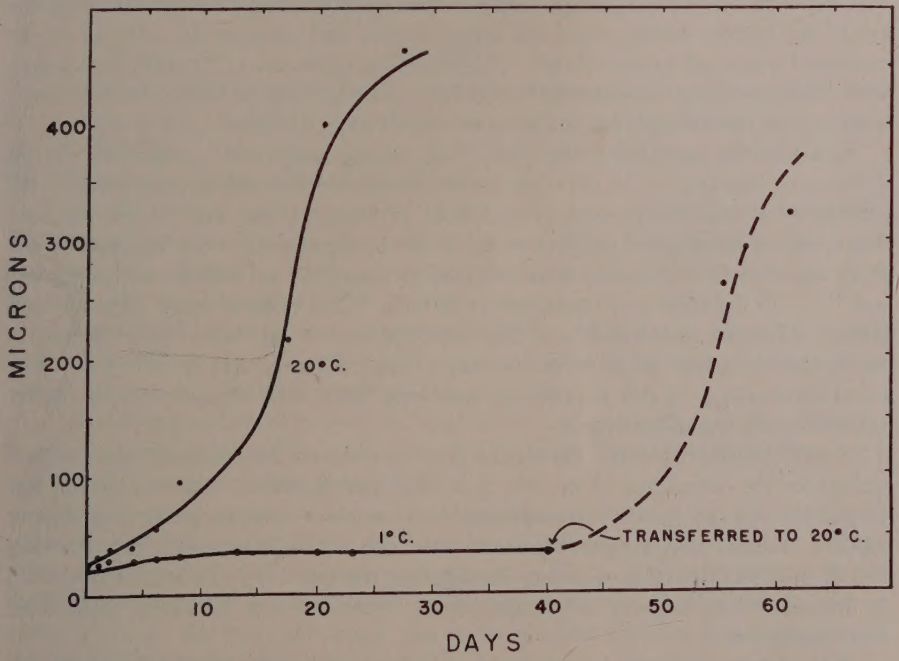
represented infections obtained during the latter part of the exposure period. Usually within a cyclops there were several larvae of about the same size. The sizes recorded represent a growth trend, rather than an absolute rate. This is due to variable factors involved such as general growth variation and stages of contraction and elongation assumed by the older larvae. The rate of growth was so markedly affected by the different temperatures that the trend shown is taken as significant. Growth was greatly inhibited at 1°C. and was best at 20°C. An erratic type of growth was demonstrated in the larvae harbored by cyclops kept at 26° and 32° C. and development did not proceed beyond 8 days. Those kept at 26° C. grew slightly up to 8 days when the larvae disappeared, while those kept at 32° C. remained at the same size or became smaller until they and the cyclops died.

To determine whether the larvae held at 1° C. for 40 days with little or no growth were permanently stunted or could undergo development under optimal con-

ditions, they were transferred to the 20° C. room. A normal growth curve resulting for these larvae between 40 and 60 days is shown in Graph II which illustrates in these animals the principle of equifinality in a steady state system.

II. Definitive Hosts

Rainbow trout, *Salmo gairdneri* Richardson and brook trout, *Salvelinus fontinalis fontinalis* (Mitchill) were found naturally infected in the reservoirs. Rainbow trout was the only species of trout used in experiments requiring a definitive host. Infection experiments were attempted with two species of warm water fish, green sunfish, *Lepomis cyanellus* Rafinesque, and bluegill, *Lepomis macrochirus macrochirus* Rafinesque, by feeding them infected cyclops. The fish were observed to feed on the copepods but no infections developed.



Graph II. The effects on growth of 1° C. and 20° C. temperatures on tapeworm larvae in cyclops, and when lower temperature was raised to 20° C. after 40 days.

Tapeworm development. Infected cyclops were fed to young trout by permitting the fish to ingest the copepods. The fish were examined for the presence of tapeworms at various intervals of time from 1 day to 11.5 months. Examinations for the 1- and 2-day infections were made by sectioning the entire alimentary tract including the liver of the fish. It was observed that 1 day following ingestion of the infected cyclops, the majority of the larvae were in the lumen of the intestine in the region of the pyloric ceca. Some tapeworm larvae were in the stomach, apparently recently escaped from the partially digested cyclops. A few were in the lumina of the pyloric ceca. Larvae ingested 2 days previously by the fish were found in the same general areas as for the day-old infections, except that none was

seen in the stomach. The young worms in the 1- and 2-day old infections measured 0.13 to 0.27 mm. in length. No evidence of tissue penetration or migration outside the gastrointestinal tract was noted.

By the 11th day of infection a few of the larvae had become established in the pyloric ceca. Others were in the lumen of the pyloric intestine. These worms were 0.33 to 0.61 mm. long. The 11-day old worms within the ceca were most frequently attached at the distal or blind end.

Worms recovered 26 days following infection were 3 to 6 mm. long. Increase in size especially in length, was the most significant change occurring in these larvae. During the 15-day period between 11 and 26 days of infection, the length of the larvae increased by 10 times. No organization of new structures was observed.

The tapeworms, recovered from fish 33 days after the fish had ingested infected cyclops, were 7 to 11 mm. long. Some indication of proglottid formation was present in the smaller forms, while the larger worms had proglottids, some of which contained testes and a cirrus pouch. Egg-expelling tapeworms (70 to 400 mm. long) were recovered after 106 days in the fish host, although worms (25 to 44 mm. long) with eggs in the uterine pouches were recovered after 63 days.

Considerable variation in size was noted among tapeworms recovered. These differences appeared to be correlated with the size of the definitive hosts and the number of tapeworms present in each fish. Another factor may be the age and degree of development of the procercoid larvae in the cyclops when ingested by the fish. The heaviest infections were obtained in those fish fed cyclops harboring larvae 15 to 19 days old and developed at 20° C. The youngest stage of procercoid larvae to become established in a fish were those of 9 days when development in the cyclops occurred at room temperature (approximately 23° to 27° C.). The infection resulting in this experiment was very light when compared with similar experiments using older larvae.

Larval transfer studies. As shown above no second intermediate hosts are required for the completion of the life cycle of *P. tumidocollus.* Since some life history studies on species of *Proteocephalus,* for example, *P. pinguis* studied by Hunter (1929), indicate that a second intermediate host, a fish, is eaten by the definitive fish host, it was desirable to determine whether the tapeworms in small trout could be transferred to larger non-infected trout. Therefore the following experiment was conducted.

Young fish were each fed approximately 8 infected cyclops which had been exposed to tapeworm eggs 18 days previously. These young fish were in turn fed to previously unexposed large trout according to the following schedule:

Hours after feeding cyclops to fish	No. fish eaten by large trout	Trout no.
18 hrs.	2	29–1
72 hrs.	1	29–2
7 days	2	29–3
15 days	2	29–3
28 days	2	29–4

Six weeks following the first feeding to large trout, the latter were examined for tapeworms. No worms were found in trout Nos. 29–1 and 29–2. Trout No. 29–3 harbored 6 tapeworms, and trout No. 29–4 had 3 tapeworms. The worms were 11 to 15 mm. long, the expected size after 6 weeks of development. This indicates that the tapeworms were established in the large fish due to eating infected fish.

Supra-infection studies. To determine the effects of delayed parasitization in fish previously infected with the same species of tapeworm, the following test was made.

Nine fish were given 54 cyclops, or an average of 6 cyclops per fish. Approximately 80 percent of the cyclops were infected with tapeworm larvae. Forty-four days later 6 of these fish were fed another 48 infected cyclops and were kept separate from the other 3. Nineteen days later, or 63 days after the initial feeding, all of the fish were sacrificed and examined for tapeworms. The results are as follows:

The 3 fish given one feeding of infected cyclops 63 days previously were infected with 1, 2, and 7 tapeworms, respectively, while no tapeworms were found in any of the 6 fish receiving the initial plus the delayed feedings of infected cyclops. It appears that some immunological factors were responsible for the removal of the parasites in the 6 fish given an initial and a delayed feeding of infected cyclops.

SUMMARY

1. Several species of copepods may serve as intermediate hosts for *Proteocephalus tumidocollus,* viz. *Cyclops vernalis, Eucyclops agilis, E. speratus* and *Tropocyclops prasinus.* Two other species of cyclops, some daphnia, ostracods and a fairy shrimp were tested with negative results.

2. Development of tapeworm larvae within cyclops was affected by temperature. The optimal temperature was about 20° C., and growth was inhibited almost entirely at 1° C. However, when cyclops harboring undeveloped larvae for 40 days at 1° C. were transferred to 20° C., normal larval growth occurred.

3. Procercoid larvae became infective to fish as early as 9 days after the cyclops were fed tapeworm eggs. The highest rates of infection in fish were obtained when cyclops infected for 15 to 19 days were fed to the fish.

4. No second intermediate host is required for the completion of the life cycle. However, when small infected fish were fed to large trout, the tapeworms were successfully transferred from fish to fish.

5. Development of tapeworms in the definitive host is gradual. The first indication of proglottid formation was 33 days after ingestion of infected cyclops. Eggs were present in the uterine pouches as early as 62 days, and were released when the worms were placed in water 106 days following infection. Egg-producing worms measured 70 to 400 mm., the larger worms having many more gravid proglottids and an increased egg producing capacity.

6. Supra-infection attempts were made by feeding infected fish additional cyclops 44 days after the first feeding. No tapeworms were found in those fish permitted to feed on infected cyclops on the 1st and the 44th day of the experiment, while tapeworms were present in the fish receiving only the initial feeding.

ACKNOWLEDGMENT

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Department, Washington, D. C., who identified the ostracods, and to Dr. N. T. Mattox, Allen Hancock Foundation, University of Southern California, for the identification of the conchostracan specimens.

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Explanation of Plates

Abbreviations used: c–cirrus; cp–cirrus pouch; h–hooklets; mi–inner membrane; mm–middle membrane; mo–outer membrane; on–onchosphere; s–sucker; sa–apical sucker; t–testis; v–vagina; vi–vitellaria.

Plate I

Larval stages of *Proteocephalus tumidocollus*

Figures 1, 2, 8, 9 and 11 were drawn with the aid of a camera lucida from living and (fig. 9) from stained material. Figures 4, 5, 6 and 7 were drawn free hand from living material. Figures 3 and 10 were traced from photomicrographs of living material.

Fig. 1. Mature egg expelled from tapeworm.

Fig. 2. Egg with onchosphere escaped from middle membrane.

Fig. 3. Recently ingested procercoid larvae in gut of cyclops.

Fig. 4. Four-day-old larva.

Fig. 5. Seven-day-old larva.

Fig. 6. Eight-day-old larva.

Fig. 7. Ten-day-old larva.

Fig. 8. Twelve-day-old larva.

Fig. 9. Thirteen-day-old larva.

Fig. 10. Sixteen-day-old larva with caudal appendage or cercomer.

Fig. 11. Naturally infected cyclops with 4 larvae which have developed beyond the cercomer stage.

PLATE I

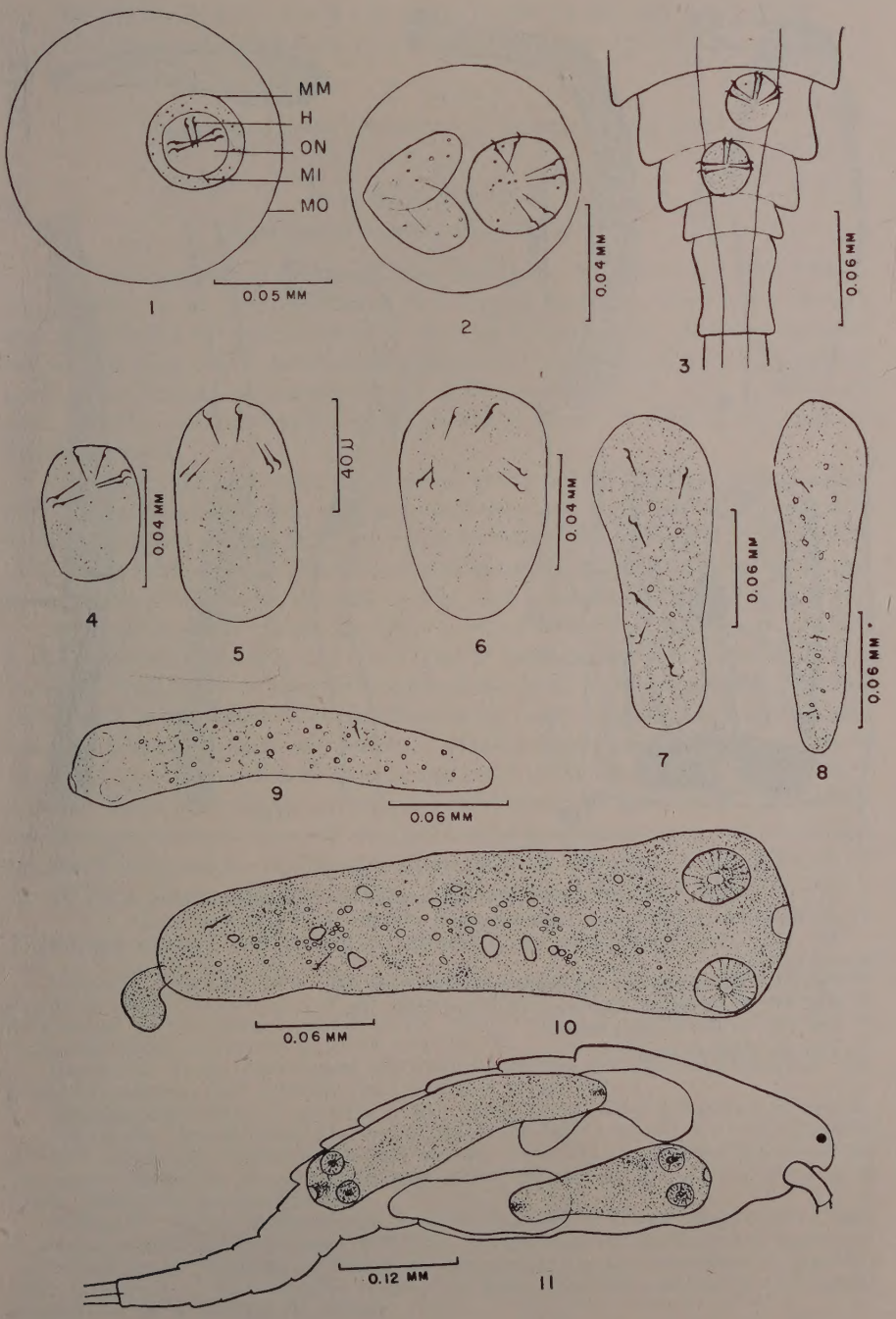
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PLATE II

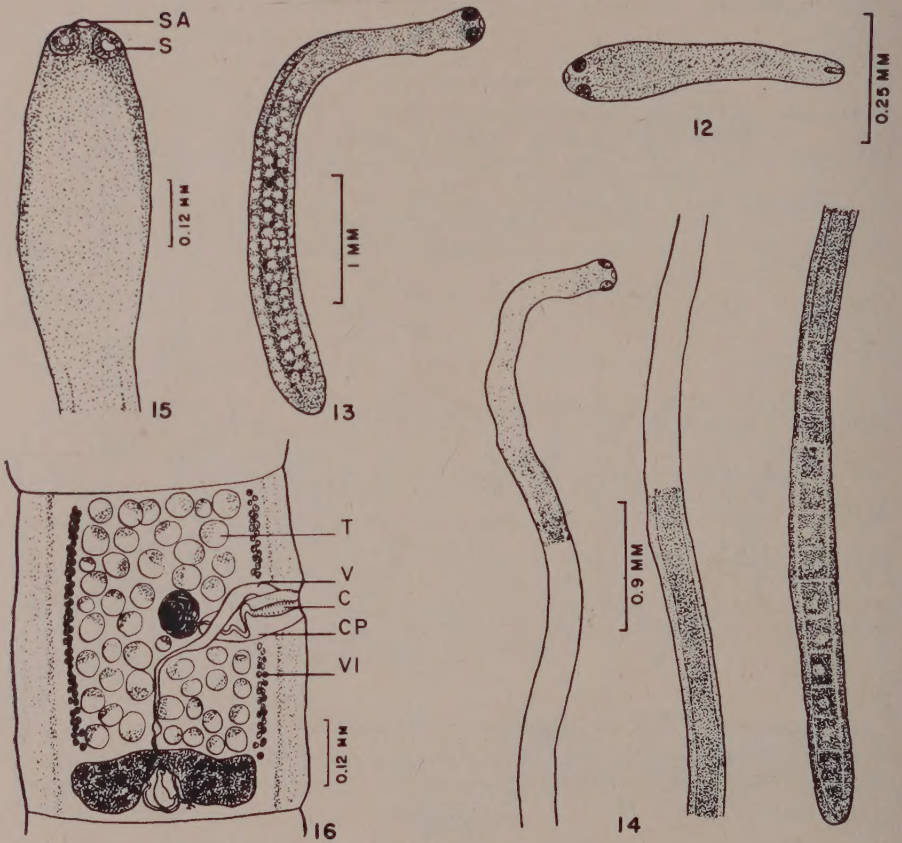

PLATE II

Young and adult *Proteocephalus tumidocollus* from rainbow trout

All figures were drawn from stained material with the aid of a camera lucida.

FIG. 12. Young tapeworm recovered from fish intestine 11 days following ingestion of infected cyclops.

FIG. 13. Twenty-six-day-old tapeworm taken from fish.

FIG. 14. Six-week-old tapeworm recovered from fish.

FIG. 15. Scolex of adult worm.

FIG. 16. Mature proglottid.

THE TREMATODE PARASITES OF THE DUGONG WITH DESCRIPTIONS OF TWO NEW MONOSTOMES AND HISTOPATHOLOGICAL CHANGES IN THE HOST

H. Crusz
Department of Zoology, University of Ceylon, Colombo, Ceylon
AND
V. S. V. Fernand
Department of Anatomy, University of Ceylon

Since the discovery of the amphistome trematode, *Indosolenorchis hirudinaceus* Crusz, 1951, in the intestinal cecum of a dugong caught in Ceylonese waters, a search was made for more material in the hope of discovering more new parasites in this important host. An excellent opportunity was afforded by the anatomical study of the dugong carried out by one of the authors (V. S. V. F.), and much fresh parasitic material became readily available, greatly facilitating its study by various methods.

Trematodes obtained from the intestinal cecum and from various regions of the stomach were studied alive as well as from pressed specimens stained in acetic acid alum carmine, and from sagittal, transverse and horizontal sections of material fixed in Bouin or Carnoy, cut 10 micra thick and stained in Delafield's haematoxylin and eosin. Stained sections of stomach nodules were made for histopathological study.

Many nematodes of the species *Paradujardinia halicoris* (Owen) were obtained from the stomach, especially from the region of the glandular pouch, and the trematode *Indosolenorchis hirudinaceus* occurred in great abundance in the intestinal cecum, 322 worms being recovered from three hosts. Besides these two species, two new trematode genera and species, *Lankatrema mannarense* and *Taprobanella bicaudata,* were recovered from various regions of the stomach, both free and encapsulated. Some observations are made on *Indosolenorchis hirudinaceus,* on the histopathological changes caused by encysted *Lankatrema mannarense,* and on the age, sex and distribution of the dugong hosts. A list of trematode species hitherto recorded from *Dugong dugong* (Müller, 1776) is also given in a table (*V. infra*).

HOSTS

Dugong I. Young specimen. Sex and length not recorded. From Jaffna Fisheries Station. Caught in Gulf of Mannar, received in Colombo, 3/6/1952. 137 specimens of *Indosolenorchis hirudinaceus* collected from intestinal cecum. Several encysted *Lankatrema mannarense* in cardiac and pyloric stomach and in pyloric ceca (Fig. 8).

Dugong II. Young female, larger than previous specimen. 6 ft. 10.5 in. long. From Achchankulam, caught in Gulf of Mannar, 28/5/1952, died in captivity.

Intestinal cecum yielded 12 specimens of *Indosolenorchis hirudinaceus,* preserved in formalin.

Dugong III. Mature female, 8 ft. long, 491 lbs. From Gulf of Mannar, received in Colombo, 8/7/1953. Nematodes (*Paradujardinia halicoris*) and trematodes (*Lankatrema mannarense* and *Taprobanella bicaudata*) in stomach. *L. mannarense* free and encysted, *T. bicaudata* free, in pyloric stomach and its pyloric ceca.

Dugong IV. Young, immature male, 5 ft. 9 in. long, 240 lbs. From Jaffna, caught in Gulf of Mannar, received alive in Colombo, 22/7/1953. 173 specimens of *Indosolenorchis hirudinaceus* in intestinal cecum. Hundreds of *Lankatrema mannarense,* free and encapsulated in glandular pouch of stomach, of which 30 were collected for study. A single specimen of *Taprobanella bicaudata* recovered from pyloric stomach.

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LANKATREMA MANNARENSE gen. et sp. nov.

(Figs. 1, 4 & 10–13)

Description: Small trematodes, broadest in middle and somewhat tapering toward ends, especially in smaller individuals. Ventral surface concave, dorsal convex. Mounted specimens 2.8–5.3 mm. long, 1.4–3.9 mm. at broadest. Cuticle smooth, without spines. Oral sucker small, muscular, almost spherical, terminal, or even subterminal due to contraction in this region; 0.16–0.34 mm. in diameter. Esophagus slender, muscular, 0.31–0.60 mm. long, 0.03–0.10 mm. in diameter, surrounded by a layer of esophageal glands, pharynx or esophageal bulb lacking. Intestinal ceca unbranched, reaching about to ovarial region, mostly hidden by extensively coiled uterus, visible in sections. Uterus occupying almost two-thirds of body. Excretory aperture at extreme posterior end. Bifurcations of excretory vesicle near pore, lateral limbs extending far forward, mediad of vitelline follicles. Testes and ovary and their ducts confined to posterior third, vitelline glands also so confined or extending at times more anteriad, but well below equator of body. Male and female genital apertures contiguous, at posterior end, in mid-line, slightly antero-ventral to excretory pore. Testes lateral, along postero-lateral margins, 0.23–0.79 mm. long, 0.13–0.37 mm. broad, deeply lobed, maximum number of lobes 7, generally only 4, lobes rarely absent and testes then ovoid. Distance of testes from posterior end depending on degree of extension of end. Vasa efferentia simple, neatly curved, meeting in mid-line to form vas deferens leading to cirrus-sac. Cirrus-sac very small, 0.11–0.17 mm. long, 0.05–0.09 mm. at broadest (proximal end or base). Pars prostatica and minute, coiled cirrus visible in sections.

Ovary large, much lobed, digitate or even dendritic; stretching from side to side, anterior to testes, spanning distance between two groups of vitelline follicles. Width of ovary 0.74–2.21 mm. Ovarian lobes directed anteriorly or laterally, posterior margin being relatively smooth. Mehlis' gland mostly post-ovarian, between testes. Oviduct slender, leading to oötype, from which leads the uterus anteriorly. Laurer's canal not seen in sections. Vitelline glands consisting of 2, somewhat extended groups of about 12 spherical to ovoid follicles, ventral, near lateral margins of body, extending from near testes to well below the equator. Follicles measuring 0.10–0.34 mm. by 0.07–0.24 mm. Yolk reservoir situated well to the right of mid-line, post-ovarian.

Uterus very slender proximally, broadening as it runs anteriorly, forming a tangled mass of ascending and descending coils occupying most of anterior two-thirds of body. At equator, or anterior to it, uterus finally swelling into a long, dorsal receptacle distended with eggs, narrowing considerably at level of ovary and proceeding as slender, thick-walled metraterm to open postero-ventrally in mid-line at female genital aperture close to male pore. Uterus throughout length packed with eggs. Eggs (Fig. 4) ovoid, operculated, 0.017–0.022 mm. by 0.008–0.010 mm., and with very long polar filament at each end. Filament at opercular end almost half the thickness of other filament, especially proximally. Filaments of immature eggs very short, growing as eggs mature, reaching length of about 0.441 mm. Slender distal ends of filaments difficult of observation, even in polarized light between crossed nicols. Therefore, maximum length of each polar filament more than 0.441 mm.

Host: Dugong dugong (Müller, 1776).

Site: Stomach (glandular pouch, cardiac stomach, pyloric stomach and pyloric ceca). (Fig. 8).

Locality: Gulf of Mannar, Ceylonese waters.

Types: Preserved material, whole mounts and stained sections of type specimens in collection of Department of Zoology, University of Ceylon.

DISCUSSION

The characters of the species described above clearly belong to the family PRONOCEPHALIDAE Looss, 1902, as emended by Ruiz (1944). The fact that the cirrus-sac and the male and female genital apertures are situated posterior to the uterus, in the posterior region of the body, indicates that the species belongs either to the NUDACOTYLINAE or to the OPISTHOTREMATINAE (see Ruiz, 1946). However, it differs from the OPISTHOTREMATINAE in the relative positions of the reproductive glands and in the fact that the uterus is extensively coiled, filling the anterior two-thirds of the body. The only feature of importance in common with the OPISTHOTREMATINAE is the median position of the genital aperture at the posterior extremity. The NUDACOTYLINAE, on the contrary, have most of the features of *Lanka-*

trema. *Neocotyle neocotyle* (subfamily: NUDACOTYLINAE), in particular, comes closest to it, in regard to the relative positions of testes, ovary, vitelline glands and uterus, and in several other minor characters. But one striking difference between them is the lateral position of the genital apertures in *Neocotyle*, which is a diagnostic character of the subfamily. But this difference cannot be considered as being of very great consequence in classification. In the PRONOCEPHALINAE and NOTOCOTYLINAE, for example, no subfamily value is attached to the position of the genital aperture, which may be median or lateral in the anterior half of the body. It seems reasonable, therefore, to assign the new species to the subfamily NUDACOTYLINAE and emend the definition of the latter. There can be no doubt that these trematodes belong to a new genus and species, for which the name *Lankatrema mannarense* is proposed (*Lanka*, Sanskrit name for Ceylon). In morphology, *L. mannarense* apparently represents a link between the NUDACOTYLINAE from the small intestine of aquatic or semi-aquatic rodents, and the OPISTHOTREMATINAE from the eustachian tubes, lungs and esophagus of the Sirenia. The following subfamily and generic diagnoses, and key to genera (modified from Ruiz, 1946) are proposed:

Subfamily: NUDACOTYLINAE

Diagnosis: *Pronocephalidae:* small; ventral surface lacking glandular structures; cephalic collar absent; posterior extremity rounded or tapering, smooth. Genital pore median or lateral, in posterior half of body, near testes. Testes, two, in posterior third. Ovary pre- or post-testicular. Mehlis' gland in front of or behind ovary. Uterus extensive, anterior to other genital organs and covering a wide field, median and lateral to intestinal ceca. Vitelline glands consisting of two groups of follicles extending along lateral margins of body, in posterior half, anterior to testes. Eggs with polar filaments. Parasites of rodents and Sirenia. Type genus: *Nudacotyle* Barker, 1916.

Lankatrema gen. nov.

Diagnosis: *Nudacotylinae* with median genital pore and pre-testicular ovary; Mehlis' gland posterior to ovary; large uterine sac preceding muscular metraterm.

Type species: *Lankatrema mannarense* gen. et sp. nov.

KEY TO GENERA OF NUDACOTYLINAE

Genital pore lateral 1
Genital pore median 2
1a. Ovary post-testicular; Mehlis' gland anterior to ovary.....*Nudacotyle* Barker, 1916
1b. Ovary pre-testicular; Mehlis' gland in zone of, and median to, ovary.
Neocotyle Travassos, 1922
2. Ovary pre-testicular; Mehlis' gland posterior to ovary; large uterine sac preceding musclar metraterm .....*Lankatrema* gen. nov.

TAPROBANELLA BICAUDATA gen. et sp. nov.

(Figs. 2, 3, 5–7 & 9)

Description: Pronocephalinae: Elongate trematodes, 6.3–12.2 mm. long, narrowest at anterior end (1.8–3.0 mm. across middle of oral sucker), broadest at posterior end, 2.2–4.2 mm. Cephalic collar distinct, though not very pronounced. Dorsal convexity and ventral concavity very pronounced, ventral surface appearing hollowed out like a boat (Fig. 3). Anterior and lateral margins infolded ventrally. Posterior end prolonged into two broad, flattened, muscular lobes, 0.62–1.57 mm. long, 1.29–2.28 mm. broad (Figs. 2, 3 & 9). Cuticle smooth dorsally, but carrying large, strong, backwardly curved spines, 0.027–0.048 mm. long, on ventral surface. Correlated with presence of ventral spines, muscle layers of ventral body-wall more highly developed than those of dorsal body-wall. Spines lacking on two posterior lobes. Oral sucker formidable, large, deep, muscular, with mouth directed ventrally and base dorsally; 1.33–1.90 mm. in diameter and 0.54–0.96 mm. deep. Esophagus narrow, 0.69–1.03 mm. long, 0.06–0.12 mm. in diameter. Pharynx and esophageal bulb absent. Intestinal ceca, provided with short branches, lateral, extending to posterior end, stopping short of posterior lobes, 3.80–7.95 mm. long,

0.24–0.59 mm. broad at posterior end. Excretory pore median, dorsal, immediately opposite ovary or slightly posterior to it. Testes, 5–8 lobed, posterior, 0.38–0.65 mm. apart and 1.41–3.09 mm. from posterior extremity, 0.29–1.06 mm. long, 0.22–0.66 mm. broad. Cirrus-sac large, elongate, thin-walled, extending from about equatorial region, or posterior to it, to the left anterior end, opening to exterior ventrally, on left side, just below mid-level of oral sucker. Cirrus-sac (Fig. 2) containing three distinct structures: coiled internal seminal vesicle forming an ovoid proximal mass, 0.29–0.40 by 0.15–0.26 mm., followed by large, darkly staining pars prostatica, 0.59–1.47 by 0.40–0.71 mm., and then long, undulating, muscular ductus ejaculatorius, attenuating as it approaches genital aperture as a cirrus. Seminal vesicle externa, large, coiled, lying immediately outside internal seminal vesicle. Ovary with 3 to 5 lobes, median, immediately behind testes, measuring 0.16–0.56 mm. by 0.12–0.52 mm. Mehlis' gland pre-ovarian, between testes. Laurer's canal not seen in sections. Vitelline glands consisting of follicles of irregular shape, distributed in two short, extra-cecal lateral fields, anterior to testes; 18–20 follicles on each side, each follicle measuring 0.034–0.162 by 0.027–0.103 mm. Uterus initially narrow, coiled near Mehlis' gland, broadening anteriorly, with coils to right and left. At base of cirrus-sac, uterus running forward only on left side of cirrus-sac, proceeding to point well below level of intestinal bifurcation, then turning on itself, running posteriorly and finally emerging midway between ovary and base of cirrus-sac as a narrow, muscular metraterm lying alongside cirrus-sac and opening at genital pore. Eggs ovoid, operculated, 0.019–0.026 by 0.010–0.014 mm., opercular end carrying 1–3 polar filaments, other end 2–3 polar filaments (Figs. 5–7). Polar filaments sometimes bifurcating some distance from base (Fig. 7). Maximum length of polar filament 0.340 mm. Polar filaments of immature eggs (Fig. 6) very short.

Host: Dugong dugong (Müller, 1776).

Site: Pyloric stomach, including pyloric ceca (Fig. 8).

Locality: Gulf of Mannar, Ceylonese waters.

Types: Type-specimens in the collection of the Department of Zoology, University of Ceylon.

DISCUSSION

The above species would definitely belong to the subfamily PRONOCEPHALINAE (family: PRONOCEPHALIDAE) if the ovary were anterior to the testes and Mehlis' gland posterior to the ovary. All other characters agree with those of the subfamily. Only two subfamilies, namely the CHARAXICEPHALINAE and the NEOPRONOCEPHALINAE, both closely related to the PRONOCEPHALINAE (see Ruiz, 1946), have a post-testicular ovary in addition to the characteristic cephalic collar. But they are as far removed from the present species as they are from the PRONOCEPHALINAE, for their vitelline glands are posterior to the testes, which, in the CHARAXICEPHALINAE, are divided into several separate follicles. Also, in the NEOPRONOCEPHALINAE, much of the uterus lies behind the testes and even behind the ovary. The possibility that this species may belong to the NOTOCOTYLINAE, in which are included species from such marine mammals as whales and seals, has also been considered. But the presence of a cephalic collar, the lack of glandular formations of the ventral surface, the fact that the posterior ends of the gut ceca do not run between the testes, and the presence of more than one filament at each pole of the eggs, point away from that subfamily. The present species, therefore, is best placed in the PRONOCEPHALINAE. About 8 genera, with widely different distinguishing characters, already belong to this subfamily, and any hesitation about placing the present species in it, on account of so unimportant a difference as a post-testicular ovary, would certainly not be justified.

The name *Taprobanella bicaudata* (*Taprobane,* Ancient Greek and Roman name for Ceylon) is proposed for this new genus and species. The following re-definition of the subfamily PRONOCEPHALINAE (modified from Ruiz, 1946) and definition of the new genus are proposed:

Subfamily: Pronocephalinae

Diagnosis: *Pronocephalidae:* medium sized, elongate body. Ventral surface without glandular formations (except *Adenogaster*). Cephalic region showing characteristic dilatation or collar. Posterior extremity rounded, truncated or bilobed. Genital pore in anterior half of body. Testes 2, at or near posterior end. Ovary pre- or post-testicular. Mehlis' gland in front of, or behind, ovary. Coils of uterus in middle third of body between gut ceca, sometimes extending laterally to or beyond gut ceca. Vitelline glands consisting of follicles situated in 2 rows at lateral margins, anterior to testes. Eggs numerous, with one to many filaments at each pole. Parasites of fishes, reptiles and mammals. Type genus: *Pronocephalus* Looss, 1899.

Taprobanella gen. nov.

Diagnosis: *Pronocephalinae.* Body boat-shaped, carrying two flattened, muscular lobes at posterior end. Ventral surface (except that of posterior lobes) armed with backwardly curved cuticular spines. Cirrus-sac large, containing internal seminal vesicle, pars prostatica, ductus ejaculatorius and cirrus. Metraterm long, muscular, lying beside cirrus-sac. Common genital aperture, ventro-lateral, at anterior end, near left margin of oral sucker. Ovary post-testicular. Mehlis' gland anterior to ovary. Eggs numerous, operculated, carrying 1–3 polar filaments at opercular end and 2–3 at other end. Excretory pore median, dorsal, at or near level of ovary. Type species: *Taprobanella bicaudata* gen. et sp. nov.

Indosolenorchis hirudinaceus Crusz, 1951

(Paramphistomatidae: Solenorchiinae).

322 trematodes of this species were collected from the intestinal ceca of 3 dugong. Some of the worms reached a length of 15 mm. Further study of serial

Table.—*Trematodes of* Dugong dugong (*Müller, 1776*)

Family and Subfamily	Species	Site of Infection
Paramphistomatidae		
Zygocotylinae?	*Zygocotyle* (?) sp. innom. Dollfus, 1950	
Solenorchiinae	*Solenorchis travassosi* Hilmy, 1949	intestinal
	S. gohari Hilmy, 1949	
	S. naguibmahjouzi Hilmy, 1949	cecum
	S. baeri Hilmy, 1949	
	Indosolenorchis hirudinaceus Crusz, 1951	
Pronocephalidae		
Pronocephalinae	*Taprobanella bicaudata* gen. et sp. nov.	stomach
Nudacotylinae	*Lankatrema mannarense* gen. et sp. nov.	stomach
Opisthotrematinae	*Opisthotrema dujonis* (Leuckart, 1874) Price, 1932	eustachian tubes and esophagus
	Pulmonicola pulmonale (v. Linstow, 1904) Poche, 1925	lungs
Rhabdiopoeidae?		
Rhabdiopoeinae	*Rhabdiopoeus taylori* Johnston, 1913.	intestine

sections of fresh material confirmed the original description (Crusz, 1951) in all details but one. It was noted that this amphistome has 3 pairs of main longitudinal lymphatic vessels: the 2 pairs originally described, namely, a ventral pair running lateral to the gut ceca and a dorsal pair median to the gut ceca, and, in addition, a third pair, also median to the gut ceca, but more ventral in position.

Histopathological Note

The presence of *Lankatrema mannarense* in the stomach of the dugong was first indicated in dugong I only by raised nodules of the mucous membrane, there being no parasites in the lumen of the stomach. The nodules contained caseous matter enclosing worm debris as shown by numerous operculated eggs with polar filaments. This led to the surmise, later proved to be correct, that the worms involved were notocotylid (= pronocephalid) trematodes. In a subsequently examined dugong, trematodes were found wriggling freely in the mucous secretion of the stomach and were also recovered from the cystic cavities that occurred under

the raised nodular areas. Some of these nodules contained caseous matter and eggs of the type seen earlier, but in some cases it was also possible to shell out whole worms from their cystic cavities. Specimens of *Lankatrema mannarense* were therefore found in 3 conditions in the dugong's stomach: viz. 1) free in the lumen, feeding on the gastric contents, especially the mucous secretions, 2) apparently encysted or encapsulated, without loss of form, as they could be shelled out easily, 3) encysted and reduced more or less completely to a caseous mass, where only eggs seem to survive longest, although, apparently, to no purpose.

These preliminary observations led to an investigation of the histopathological changes, if any, brought about by the trematodes as a result of their penetration of, and seeming encystment in, the stomach tissues of the host. For this purpose portions of stomach-wall were serially sectioned and stained in Delafield's haematoxylin and eosin, or in Mallory's triple stain. Microscopic examination revealed histological pictures (Figs. 11–13) that can, for convenience, be treated as representing 3 different conditions, although probably they are but stages in one continuous process in the cystic life of these trematodes.

These stages are: a) One or two worms are seen to lie in cystic spaces of invaginated mucous epithelium, below which the muscularis mucosae and submucosa are considerably thinned out (Figs. 11 & 12). Each space is lined by cuboidal epithelium continuous with the gastric epithelium. It would appear, from the cuboidal nature of the cystic epithelium, that the worms when quite young had invaded a gastric gland and grown there, enlarging it to cystic proportions. This cystic epithelium would not, therefore, correspond to that seen in typical epithelization around foreign bodies penetrating skin or gut epithelium (Pflugfelder, 1950). The histology of the worms themselves shows that they were alive at this stage. In fact, in some instances, worms can be seen protruding from a minute opening in the mucosa, leading to the cavity in which they lie (Fig. 11). Correlated with this observation is the fact that there are no signs of cellular reaction on the part of host tissue as obtains in typical foreign body invasion. There is no infiltration of submucosa with lymphocytes, eosinophiles or plasma cells. There seems to be only an alteration in the staining reaction of the connective tissue which immediately surrounds the cystic epithelium and may represent modified muscular tissue.

b) In many cystic spaces, however, the worms are obviously dead and almost completely unrecognizable but for the very typical eggs. A cyst of this type (Fig. 13) consists of a large mass beneath the mucosa, encroaching on the submucosa, but superficial to the actual muscular coat of the stomach (muscularis). This mass contains, in the center, remnants of a worm (only eggs and lining of uterus are to be clearly seen) in addition to necrotic debris infiltrated with a few polymorphs and mononuclears. The wall or lining of this mass is composed of large collections of plasma cells, lymphocytes and giant cells surrounded by a zone of incipient fibrosis, which later increases considerably. The whole picture is one of dense reaction of the tissues, of the nature of a foreign body reaction.

c) The third and last type of histological picture observed is that of a very large caseous mass (3 to 4 times the size of that described above) containing necrotic debris and organisms. This mass is separated from the adjoining tissues by a zone of cellular reaction composed of plasma cells, lymphocytes, mononuclears, giant cells and fibrotic tissue. Worm traces are almost completely absent, but for

a few eggs that can be recognized here and there in the central mass. The large size of this type of caseous mass can probably be attributed to the confluence of a number of closely situated cystic spaces containing many dead worms.

From this histological study three facts stand out clearly: 1) No cellular reaction takes place around any of the worms which are apparently alive in the cystic spaces. 2) Intense cellular reaction in submucosa occurs only around worms that for some reason have died. 3) Cystic spaces containing worms, dead or alive, do not encroach on the muscular coat (muscularis) of the stomach.

Clear as may be the histological pictures just described, exactly how they have been caused is not so easy of explanation. One explanation may be that these small trematodes, in an early stage of their life in the stomach, invade gastric glands by way of exploration, enlarge them as they grow larger and live in them for some time at least, most of the worms probably wriggling out later. A deeper ingress into and a longer sojourn in host tissues may bring about a complete closure of the original outlet from the cystic cavity and, as a result, conditions unfavorable to the parasite could soon develop and cause its death. Thereafter, the parasite would act as a foreign body and provoke rapid cellular reactions resulting ultimately in the complete caseation of the worm elements. The cystic space that once contained one to many worms, or many such spaces by confluence, then may become consolidated into a hardened nodule containing only caseous matter and, at most, a few recognizable trematode eggs.

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PLATE I

FIG. 1. *Lankatrema mannarense* gen. et sp. nov. Ventral view.
FIG. 2. *Taprobanella bicaudata* gen. et sp. nov. Ventral view.
FIG. 3. *Taprobanella bicaudata*. Drawing of freshly fixed specimen.
FIG. 4. Egg of *Lankatrema mannarense*.
FIGS. 5–7. Eggs of *Taprobanella bicaudata*.
FIG. 8. Stomach of *Dugong dugong*, showing various regions. A. Opening of esophagus into stomach. B. Glandular pouch. C. Cardiac stomach. D. Pyloric stomach. E, E. Pyloric ceca.

PLATE II

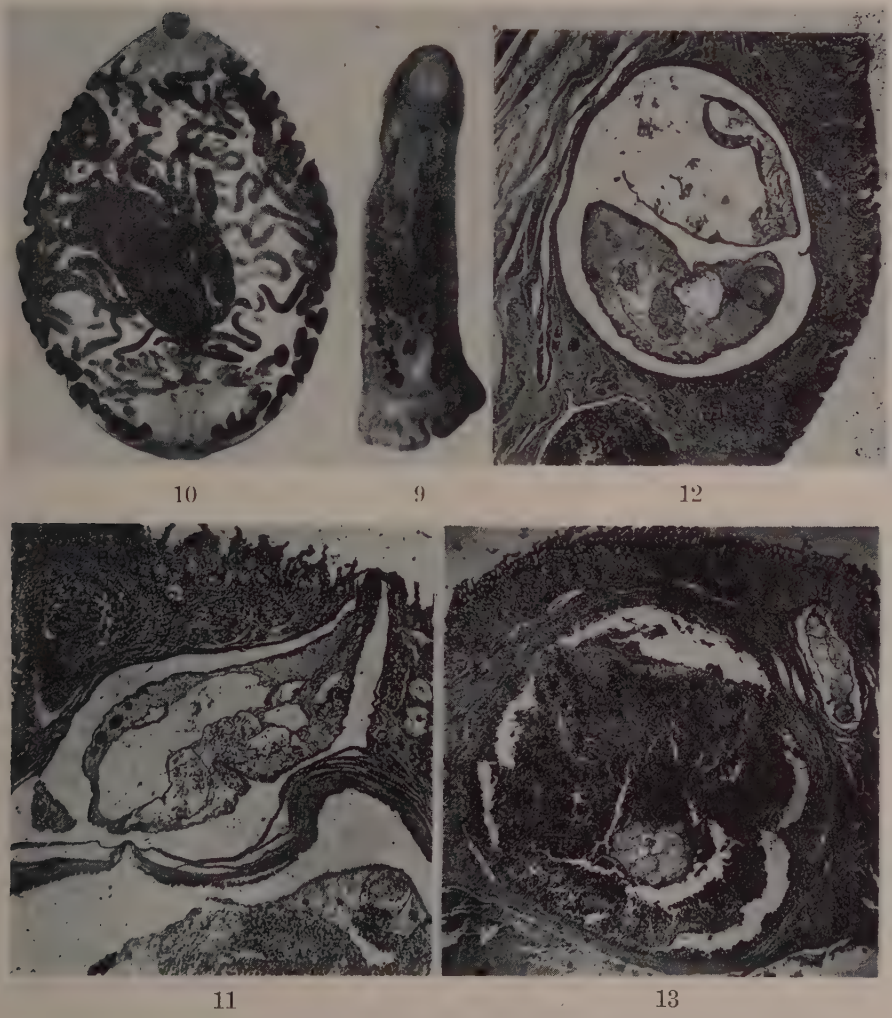

FIG. 9. *Taprobanella bicaudata.* Photomicrograph of stained, mounted specimen. ×4¾.

FIG. 10. *Lankatrema mannarense.* Photomicrograph of stained, mounted specimen. ×12.

FIG. 11. Portion of stomach-wall of dugong, showing *Lankatrema mannarense* in cystic cavity presumably formed of invaginated gastric epithelium. Note anterior end of worm protruding into lumen of stomach through opening in cystic cavity. Note also absence of cellular reaction around cavity. Serial section, 10 microns thick, stained in Delafield's haematoxylin & eosin. Photomicrograph ×32.

FIG. 12. Portion of stomach-wall of dugong, showing two specimens of *Lankatrema mannarense* in same cystic cavity. Serial section, 10 microns thick, Delafield's haematoxylin & eosin. Photomicrograph ×32.

FIG. 13. Portion of stomach-wall of dugong, showing dense cellular reaction, of the nature of a foreign body reaction, around dead, encapsulated worm of species *Lankatrema mannarense.* A large fragment of the worm, containing eggs, can be seen in the center of the mass. Serial section, 10 microns thick, stained in Delafield's haematoxylin & eosin. Photomicrograph ×20.

CHARACTERISTICS OF *HEXAMASTIX BATRACHORUM* (ALEXEIEFF)

BRONISLAW M. HONIGBERG AND HOWARD H. CHRISTIAN[1]
Department of Zoology, University of Massachusetts, Amherst

INTRODUCTION

During an extensive survey of the intestinal flagellate fauna of amphibians which the senior author has conducted for the last several years he found *Hexamastix* in only 1 salamander and failed to find it in any frogs or toads. As far as can be ascertained, there are only a few records of this trichomonad genus from amphibians. Most of them deal with *Hexamastix batrachorum* (Alexeieff) from salamanders. Wenrich (1940) mentioned *Hexamastix* sp. from frogs, but his brief note, which dealt primarily with parasites and inclusion bodies in protozoa, contained neither description nor figures of this flagellate. In a personal communication, Dr. Wenrich informed us that he had found *Hexamastix* in a few frog tadpoles. The fact that the survey made by one of us included only a very small number of tadpoles may explain the failure to find any members of this flagellate genus in anurans. Dr. Wenrich suggested that while there was no reason to suppose that the organism he found in tadpoles was not *H. batrachorum*, a positive identification of this flagellate must be based on further study of his material. Thus it is evident that, until such a study is made, or until additional anuran hosts that harbor *Hexamastix* are found, *H. batrachorum* has to be considered as restricted to salamanders.

All the data derived from the available literature, from the personal communication of Dr. Wenrich, and from the aforementioned survey indicate that *Hexamastix* is among the rarest inhabitants of the intestine of amphibians.

For reasons set forth in a later part of this paper, we decided that the flagellate we found in the salamander was *Hexamastix batrachorum* (Alexeieff).

While lizards harbor at least 2 morphologically distinct trichomonads that belong in the genus *Hexamastix*, neither of these organisms can be placed in the same species with the congeneric flagellates of salamanders.

The material studied by us showed many hitherto undescribed characters of *H. batrachorum* and provided an excellent source of information concerning the structure of the genus *Hexamastix* in general.

MATERIALS AND METHODS

Among 6 spotted salamanders (*Ambystoma maculatum*) collected in a single locality and examined during the spring of 1951, 1 was found to harbor *Hexamastix batrachorum*. Additional hosts belonging to the same species were collected in the same and other localities, but none of them was infected with this flagellate. A number of tiger salamanders (*Ambystoma tigrinum*), the intestinal flagellate fauna of which is very similar to that found in *A. maculatum*, proved to be negative for *Hexamastix*.

Thin wet smears of the contents of the large intestine were fixed in either Bouin's

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or Schaudinn's fluids. Preparations fixed in Bouin's fluid were stained with protargol according to modified Bodian's method (see Honigberg, 1951), or with iron hematein (or hematoxylin). Fixation in Schaudinn's fluid was followed by iron hematein (or hematoxylin) staining only.

DESCRIPTIVE ACCOUNT

(All measurements are in microns)

The body is ellipsoidal or ovoidal in shape (figs. 1–3, 5). Occasionally more nearly pyriform organisms are found (fig. 4). The body ranges in length from 7 to 15.5, in width from 5 to 10.5, averaging 11×7.

There are five unequal (fig. 2) or nearly subequal (fig. 4) anterior flagella which originate in the anterior part of a large basal granule complex (fig. 1). The flagella are usually united in a short whip or column at their base (figs. 1, 3–6) and terminate in rods or knobs (figs. 2–5). In some specimens the flagella are separate from the point of their origin. The longest anterior flagellum measures 20(15.5–23.5).

Counts of the anterior flagella were made in 100 random organisms on one slide; 63 specimens had five, 11 specimens four, and 1 specimen three. The remaining 25 organisms were unsuitable for definite counts. Judging from the large size of 7 of the specimens with four anterior flagella, and of the single 1 with three flagella, it is likely that in these organisms some of the flagella were lost in the process of making the preparation. Since no divisions were observed in the population that we studied, this population could be expected to contain only a few young organisms in which the full flagellar complement has not yet been restored. Nevertheless the 4 smaller specimens with four anterior flagella undoubtedly represented younger individuals[2].

The recurrent flagellum originates in the dorsal part of the basal granule complex (figs. 1–3, 6), and measures in length 21(15–28), being thus slightly longer than the anterior flagella. As in many other trichomonads, it ends in a short slender filament (figs. 3, 5).

The "V"-shaped parabasal apparatus, situated to the right of the nucleus, originates in the right part of the basal granule complex (figs. 1–5, 7). The arms of the parabasal body, usually of about equal length, widen gradually from the proximal towards the distal end, being most often nearly triangular in shape (figs. 3–6, 9). They are laterally flattened and may exhibit folds and twists (figs. 9, 10). The arms often stain very lightly with protargol, particularly in their distal parts. In some side views both arms appear as rods of uniform width (fig. 2). Only a few specimens show the parabasal filaments, which may accompany either the dorsal or the ventral arms (figs. 2–4). However, in view of the situation prevailing in other trichomonads that have a "V"-shaped parabasal apparatus, it is likely that also in this species the organelle contains two filaments. Iron hematein (or hematoxylin) is unsuitable for demonstration of the parabasal apparatus. Whenever it can be seen in preparations thus treated, the parabasal appears to consist of two darkly staining filaments (fig. 1).

The hyaline axostyle originates in the vicinity of the basal granule complex with which it is connected (figs. 1–3, 6). The anterior part of the axostyle is ex-

[2] The development of the mastigont structures in a species of *Hexamastix* from reptiles is described in detail by the senior author in a paper which is now in press.

panded into a spatulate capitulum that is closely applied to the left ventro-lateral surface of the nucleus and extends for some distance posterior to it (figs. 2, 4, 6). The trunk of the axostyle, usually a slender rod (about 0.3 in diameter), runs through nearly the center of the body and projects from its posterior end (figs. 1–3, 5). The posterior projection averages 8(3.5–12) in length, and tapers gradually to a point. Occasionally the trunk of the axostyle is somewhat more robust (fig. 4). The axostyle contains no granules, nor is it surrounded by periaxostylar rings.

The pelta is a very conspicuous crescent-shaped membrane situated near the anterior surface of the body (figs. 1–7, 9). It curves from the right-dorsal towards the left-ventral side of the organism, ending near the left-ventral surface of the nucleus (figs. 2, 3, 5, 7). In some specimens the pelta exhibits undulations and folds (figs. 4–6). The membrane remains in a close spatial relationship with the anterior part of the axostyle (figs. 2–4, 6) and probably constitutes a development of the capitulum. The pelta is seen not only in preparations impregnated with protargol, but also in most organisms stained with iron hematein (fig. 1).

None of the hitherto studied members of the families MONOCERCOMONADIDAE and TRICHOMONADIDAE (*sensu* Kirby, 1947) possess a pelta that is relatively as large as the one found in *Hexamastix batrachorum*. The membrane, as seen in this species, resembles very much the probably homologous structures associated with the axostylar capitulum in certain devescovinids (for example *Metadevescovina*).

The nucleus, situated about 2 microns below the anterior surface of the body, varies in shape from broadly ellipsoidal or ovoidal (figs. 1–3, 5) to nearly spheroidal (figs. 4, 6). In almost every organism in the population that we studied, the chromatin content was drawn away from the nuclear membrane forming a central darkly-staining mass. The nuclei contain a single nucleolus and measure 3(2–3.5) by 2.5(1.5–3).

DISCUSSION

Alexeieff (1909) reported a six-flagellate trichomonad from the large intestine of the salamander *Triton taeniatus* (=*Triturus vulgaris*). He failed to name the organism, but recognized its relationship to *Trichomastix* (=*Monocercomonas*). Alexeieff (1911) placed this flagellate in the genus *Polymastix* Bütschli as a new species *batrachorum*; later, however, he (Alexeieff, 1912) realized that it was not congeneric with other species of the genus *Polymastix*, and therefore established a new genus *Hexamastix* for the trichomonad. On the basis of the descriptions given by him on several consecutive occasions (Alexeieff, 1909, 1911, 1912, 1914b, 1917), we may formulate the following diagnosis of the species: "body more or less pyriform, averaging 8–14 microns × 4–8 microns; six unequal flagella which adhere to one another for some portion of their length; axostyle well developed, usually projecting from posterior end of body; nucleus spherical with eccentric karyosome, near anterior end of body; a more or less complete ring or crown of perinuclear granules."

It should be noted that this diagnosis fails to differentiate between the anterior and the recurrent flagella.

In his description of the intestinal protozoan fauna of the horse leech, *Haemopis sanguisuga*, Alexeieff (1914a) included an organism which, according to him, was identical with *Hexamastix batrachorum*. As pointed out by Grassé (1926), it seems improbable that a salamander and a leech harbor the same species of parasite. Thus,

pending further investigation of the protozoan fauna of the horse leech, Alexeieff's specific diagnosis of *Hexamastix* from this host must be approached with caution.

Grassé (1926) reported *Hexamastix batrachorum* from the salamander, *Molge palmata* (=*Triturus helveticus*). In most details his description agreed with that given by Alexeieff. In addition, Grassé differentiated between the anterior and the recurrent flagella, and stated that the five anterior flagella were very unequal. Furthermore, he described "inconstant" basophilic granules in the slender axostyle and the parabasal body which appeared as a "skullcap" applied to the anterior surface of the nucleus.

In his note on the intestinal protozoa of the larvae of the crane-fly, *Tipula abdominalis*, Geiman (1932) listed, under the name *Hexamastix batrachorum* var. *tipulae*, an organism which, except for a "more developed axostyle," exhibited all the characters of *H. batrachorum*. Ludwig (1946), after a more thorough investigation of the protozoan fauna of the crane-fly larvae, reported that the six-flagellate trichomonad from these hosts differed from *H. batrachorum* in that it had a "thinner" axostyle and lacked the parabasal body. He proposed a new combination *Hexamastix tipulae* for this organism. While it is doubtful that any trichomonad species actually lacks the parabasal apparatus, the difference in the diameter of the axostyle and the fact that one of the flagellates comes from vertebrate and the other from invertebrate hosts favor the opinion expressed by Ludwig. A study based on observations of living organisms with the aid of phase contrast and of protargol-treated preparations would probably bring out additional morphological differences between the two species.

The organism that we studied from the spotted salamander fits in well with the diagnoses given by Alexeieff and Grassé. The fact that the anterior flagella in the specimens we observed were not pronouncedly unequal in length may be due to the relative stability of the population in which no divisions were observed. Since the axostylar granules were not reported at any time by Alexeieff in *H. batrachorum*, and since Grassé referred to them as being "inconstant," their absence from our material is of little taxonomic significance. Alexeieff never described the parabasal apparatus in this species with any accuracy and Grassé described and figured it as resembling a "skullcap." Both of these workers made their observations primarily from iron hematoxylin-stained material, a method often inadequate for demonstrating the details of this organelle. It is therefore not surprising that our study of preparations stained with protargol revealed what is, in all probability, a truer picture of the parabasal. In addition, all specimens thus stained showed an exceptionally well-developed pelta. The pelta can be seen also in iron hematoxylin-stained preparations, but since the earlier authors lacked experience with that structure, which may best be studied in protargol-impregnated organisms, they could easily have overlooked it.

It is well established that many members of the family Monocercomonadidae possess in the anterior part of the body a well-defined and often rather typically arranged mass of perinuclear or frontal granules which stain with hematoxylin, and which may be seen clearly in living specimens observed with the aid of phase contrast. Alexeieff (1917) and Grassé (1926) reported the presence of such granules in *H. batrachorum*. However, when no examination of living material is made, and all observations depend upon a limited number of fixed and stained preparations, one

may fail to obtain a true picture of these structures. This might have been true with our material. It should be noted that none of the figures of *H. batrachorum* included in the papers of either Alexeieff or Grassé shows these granules clearly.

All previous authors described the nucleus of *H. batrachorum* as being spheroidal. In some of the specimens we examined the nuclei were spheroidal, but more often they were very broadly ellipsoidal or ovoidal. However, after observations of many species of trichomonad flagellates in living state with the aid of phase contrast, one cannot attach too much importance to the exact shape of the nuclei, which in most instances are relatively plastic.

On the basis of the foregoing arguments we feel quite certain that the flagellate we found in the spotted salamander belongs in Alexeieff's species *H. batachorum.*

TAXONOMIC SUMMARY

Hexamatix batrachorum (Alexeieff)

Polymastix batrachorum Alexeieff, 1911, Arch. Zool. Exper. et Gén., **6:** 495, textfig. 3, *a, b.*
Hexamastix batrachorum (Alexeieff, 1911), Alexeieff, 1912, Zool. Anz. **39:** 679.
Type host.—Triturus vulgaris (Linné) [= *Triton taeniatus* (Schneider)]. Europe. (Alexeieff, 1909, 1911).
Additional hosts.—
Triturus helveticus (Razoumowsky) [= *Molge palmata* (Merriam)]. Near Montpellier, France. (Grassé, 1926).
Ambystoma maculatum (Shaw). Near Hawley swamp, Cushman, Hampshire Co., Massachusetts. Hyposyntype slides GP-163: 1, 3, 7, 8.

Diagnosis (from *Ambystoma maculatum*): Body usually ellipsoidal or ovoidal, measuring 11(7–15.5) microns × 7(5–10.5) microns; in fully developed organisms unequal or nearly subequal anterior flagella ending in knobs or rods, longest flagellum measuring 20(15.5–23.5) microns in length; recurrent flagellum ending in short slender filament, measuring 21(15–28) microns in length; parabasal apparatus "V"-shaped, near right side of nucleus, arms of parabasal body laterally flattened, nearly triangular in shape; axostyle hyaline; capitulum of axostyle flattened expansion, applied to left ventro-lateral surface of nucleus; trunk of axostyle slender rod, about 0.3 micron in diameter, projects from posterior end of body for 8(3.5–12) microns, projection tapers gradually to a point; pelta very large crescent-shaped membrane near anterior surface of body; nucleus variable in shape, broadly ellipsoidal or ovoidal or nearly spheroidal, about 2 microns below anterior surface of body, measures 3(2–3.5) microns × 2.5(1.5–3) microns.

SUMMARY

Hexamastix batrachorum, one of the rarer intestinal flagellates of amphibians, was found in the spotted salamander, *Ambystoma maculatum.*

The body of the flagellate is usually broadly ellipsoidal or ovoidal. The mature organisms are characterized by five unequal or nearly subequal anterior flagella and a slightly longer recurrent flagellum. The parabasal apparatus is "V"-shaped, the arms of the "V" being laterally flattened and nearly triangular. The capitular expansion of the hyaline axostyle is applied to the left ventro-lateral surface of the nucleus. The trunk of the axostyle is a slender rod which, after passing through the body, projects from its posterior end and tapers gradually to a point. The pelta is very large. The nucleus, which contains a single nucleolus, varies in shape and is broadly ellipsoidal or ovoidal, or else nearly spheroidal.

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Explanation of Plate

All figures are of *Hexamastix batrachorum* (Alexeieff) from *Ambystoma maculatum*. They were made with the aid of camera lucida, at the original magnification of × 4200, from preparations fixed in Bouin's fluid. Unless stated otherwise, the preparations were stained with protargol. The figures were reduced in print by about one-third.

Abbreviations: a. fl., anterior flagella; ax., axostyle; pb., parabasal body; pe., pelta; r. fl., recurrent flagellum.

Fig. 1. Dorsal view of an iron hematein-stained organism. The parabasal body is represented by slender filaments which originate in a large basal granule. The nuclear detail shows clearly.

Figs. 2, 3. Dorsal views. The arms of the parabasal, one of which is associated with a fine filament, appear to be of uniform width in fig. 2, but are more typically shaped in fig. 3. The capitulum of the axostyle is seen clearly in fig. 2.

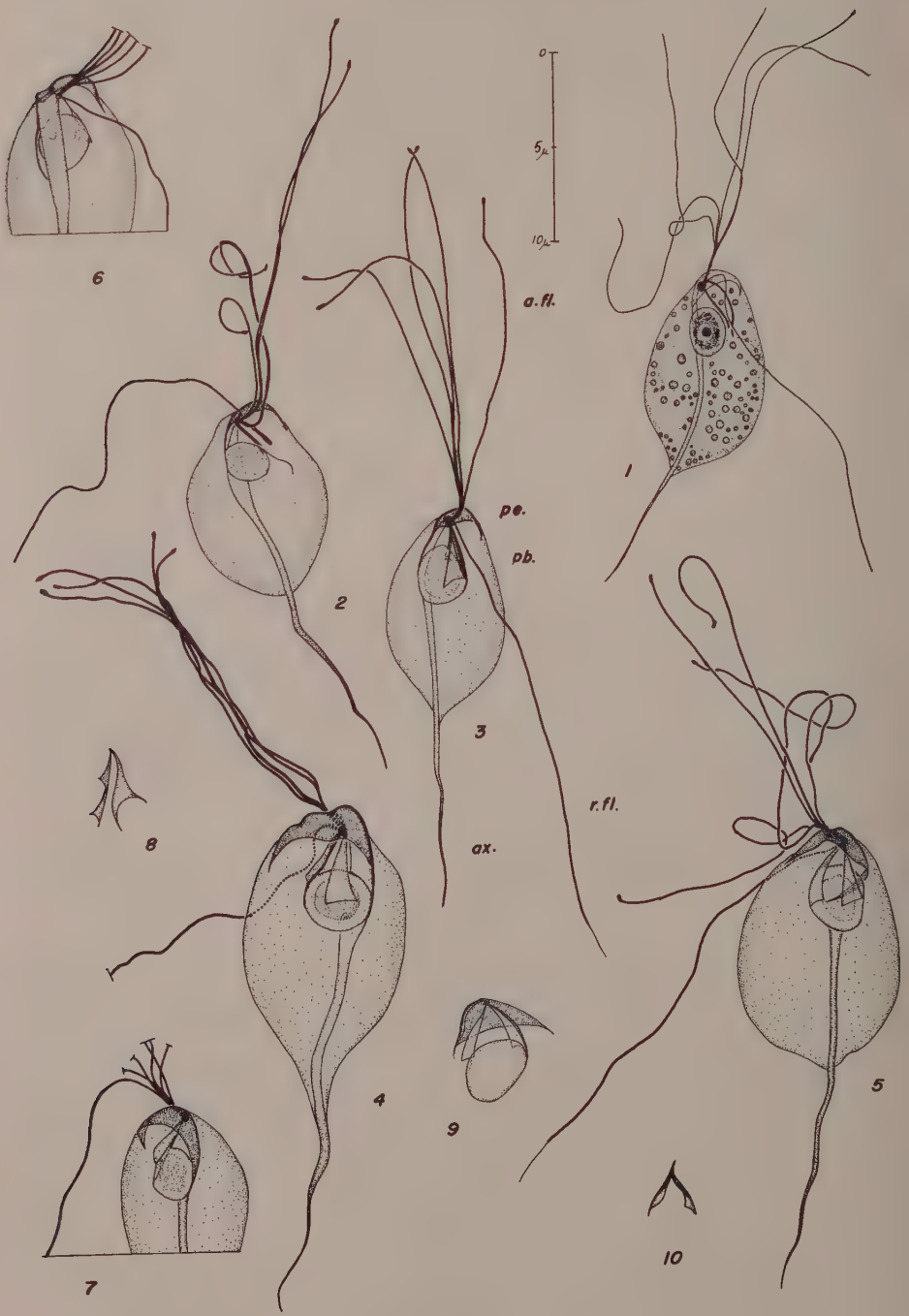
Figs. 4, 5. Right views. In both specimens the typical parabasal apparatus is situated to the right of the nucleus. The peltas are well developed and show folds and undulations. The nucleus is nearly spheroidal in fig. 4.

Fig. 6. Left view of the anterior part of the body. Note the capitular expansion of the axostyle and the pelta which covers the anterior end of the capitulum.

Fig. 7. Ventral view of the anterior part of the body.

Figs. 8, 9, 10. Diagrams of the parabasal bodies. In fig. 9 the diagram includes the left view of the pelta and of the nucleus.

PLATE I



AUSTROBILHARZIA VARIGLANDIS (MILLER AND NORTHUP, 1926) PENNER, 1953, (TREMATODA: SCHISTOSOMATIDAE) IN HAWAII WITH NOTES ON ITS BIOLOGY*

GEORGE W. T. C. CHU AND CHARLES E. CUTRESS
Department of Bacteriology and Department of Zoology,
University of Hawaii

INTRODUCTION

In November, 1950, apharyngeal, furcocercous cercariae were recovered from marine snails (*Littorina pintado* Wood) collected from the rocky shores of two bird islands just off the coast of Oahu in Hawaii. Chu (1952) reported the cercariae and the possibility of their being the etiological agent involved in local cases of "swimmers' itch." Subsequently, young noddy and sooty terns (*Anous stolidus pileatus* Scopoli and *Sterna fuscata oahuensis* Bloxam), chickens and ducks were experimentally exposed to these cercariae, and, as a result, sexually mature schistosomes were recovered from mesenteric veins in birds of all these species. All stages of the life cycle have been successfully maintained in the laboratory for more than 1 year. In December, 1952, adult worms identical with those raised experimentally were recovered from the mesenteric veins in 4 out of 8 ruddy turnstones, *Arenaria interpres interpres* (L.), killed on the island of Oahu. A careful comparison of the morphology of the adults and larval stages of the Hawaiian schistosome with that of other species in the subfamily SCHISTOSOMINAE, as far as published data have permitted, shows that the Hawaiian worm most closely resembles *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951.

However, the Hawaiian schistosome differs from *Microbilharzia chapini* Price, 1929, which Stunkard and Hinchliffe (1952) reduced to synonymy with *M. variglandis*. After comparing the description and figures of *M. variglandis* with the type of *M. chapini*,[1] it is hard to reconcile the striking difference between their ceca, which are straight and without diverticula in *M. chapini* but anastomosing and with numerous diverticula in *M. variglandis* (and in the Hawaiian schistosome). The configuration of this organ is so constant in the Hawaiian worms that it appears to rate consideration as a specific character. In addition, a comparison of cecal measurements for the 2 species, based on published descriptions (E. W. Price, 1929; Stunkard and Hinchliffe, 1952), shows that the common cecum of *M. chapini* is only about one-third the length of that in *M. variglandis*.

Penner (1953) suppressed the genus *Microbilharzia* and reallocated the species to the genus *Austrobilharzia* Johnston, 1916. He is probably correct in doing so, but such a change would be more justified had a rather complete morphological study of both the adult and larval stages of *Austrobilharzia terrigalensis* been made.

Since rather complete reviews of the literature on the avian schistosomes have been included in several recent papers, *e. g.* Stunkard and Hinchliffe (1952), no

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[1] The authors wish to thank Dr. E. W. Price, National Museum, Washington, D. C., for the loan of the type specimen of *M. chapini* for comparative study.

review is included in this report. In view of the fact that several aspects of the adult and intermediate stages of *Austrobilharzia variglandis* have not been adequately described, the following additional observations on the morphology of the adults, the development of larval stages, as well as the mode of infection in both the definitive and intermediate hosts are offered.

THE ADULT STAGE

The adult Hawaiian schistosomes from both the ruddy turnstone and experimentally infected birds agree in most respects with the species described by Stunkard and Hinchliffe (1952). The dimensions of the more than 100 Hawaiian worms measured are, however, preponderantly greater than those recorded by these workers for their species. Our measurements were made from living worms after relaxation in a refrigerator, and from specimens compressed between cover glasses and fixed in Lavdowsky's fluid. One group of about 20 worms was measured before and after fixation, and it was found that the method employed in flattening our specimens so nearly compensated for any shrinkage during fixation that there was no appreciable difference in measurements of length and width between living and fixed worms. The few specimens that were observed to contract strongly or become contorted at the time of fixation were discarded. Since no obviously contracted or contorted worms were measured, we believe that the greater length and width of the Hawaiian species can be attributed largely to the condition of the specimens measured, although it may also be due, in part, to differences in their degree of maturity.

The sexually mature male Hawaiian worms (Fig. 1) measure 4.0–6.0 mm. by 0.28–0.56 mm. from natural infections and 4.0–7.0 mm. by 0.20–0.70 mm. from experimental infections. According to Stunkard and Hinchliffe, the Atlantic Coast species measures 2.94–4.22 mm. by 0.46 mm. In the Hawaiian species the gynaecophoric canal begins at the oral sucker, and is lined with spines arranged in transverse bands about 13 microns wide separated by smooth areas of about 32 microns. The spines become much reduced in the preacetabular region. The canal cephalad of the acetabulum is very apparent in living specimens but indistinct in most preserved worms, which may account for Stunkard and Hinchliffe's placing the origin of the canal at the acetabulum of their worms. The fact that Stunkard and Hinchliffe (1952) figure the acetabulum to be within a Y-shaped "origin" of the canal strongly suggests that the canal actually does begin at the oral sucker in their specimens. If it originated at the acetabulum, the folds forming the canal would arise as an inverted V or as a line rather than as a Y. The number of testes varies with age. Spermatogenesis begins in the most posterior testis and gradually progresses cephalad as the more anterior primordia undergo organogenesis. Worms 2 weeks old generally possess 13 testes, those more than 4 weeks old average 20, and a few 4 months old have 23, the maximum number observed in Hawaiian worms. The excretory system in the male consists of 2 ventro-lateral protonephridia, whose main ducts arise just caudad of the oral sucker and unite just before the terminal excretory pore to form the definitive bladder. In the male (but not in the female) there is an accessory duct on each side which arises near the level of the beginning of the terminal cecum and joins the main duct a short distance cephalad of the definitive bladder. The flame cells occur binately in a bilateral

series, and most are lateral to the main protonephridial ducts. There are 4 lateral binary groups of flame cells along each accessory duct and 3 lateral and 2 medial binary groups along the terminal portion of each main duct. The capillaries from each binary group unite and in common join the ducts at rather constant intervals of about 50 microns. Not all the flame cells were visible in any one of the many worms examined, but those few flame cells obscured by other internal organs in 1 worm were seen clearly in one or more other specimens. The estimated number of flame cells for an average adult male (6 mm. long), based on direct observation and on calculations from their periodicity along the ducts, is approximately 280.

Sexually mature female schistosomes from Hawaii (Fig. 4), recovered from experimental infections, measure 3.0–7.0 mm. by 0.20–0.30 mm. in comparison with 2.0–3.5 mm. by 0.05–0.175 mm. recorded for female worms from the Atlantic Coast. Many authors dealing with schistosomes have regarded the number of eggs in the uterus as important. In mature females from Hawaii one egg is almost always found in the oötype, and a few worms have had 6 to 8 female germ cells scattered along the length of the long oviduct. However, only on rare occasions have eggs been found in the short uterus, which indicates that they probably pass through this organ rapidly. The excretory system of the female differs from that of the male in the more simple arrangement of the ducts in the region of the definitive bladder and in the shape of the bladder, which is trigonal in the female but cylindrical in the male. The protonephridial ducts arise immediately behind the oral sucker and unite posteriorly to form the definitive bladder. Most of the flame cells are lateral to the ducts, but occasionally some shift to a medial position. In the female the capillaries from the binary groups of flame cells unite and then join the ducts at about 85 micron intervals. The total number of flame cells in an average sized female (6 mm. long), estimated on the same bases as for the male, is approximately 164.

In all infected birds examined, the adult worms were usually recovered from the mesenteric veins adjacent to the ileum, but occasionally, in heavy experimental infections, a few worms were also taken from pancreatic veins and veins near the cloaca. On 2 occasions, involving repeatedly infected terns, 20 to 30 adult worms were recovered from veins in the liver. All natural infections were light, not more than 4 pairs of worms being recovered from 1 bird. Single and repeated infections of chickens and terns, although initiated by exposing the birds to more than 500 cercariae, have never yielded more than 60 males. However, in these infections polygamy was the rule, and 3 to 8 females occupied the gynaecophoric canal of most males. When many females occupied a gynaecophoric canal, some were small and retarded in development. On the contrary, bachelorhood in unisexual infections did not seem to alter the size of the male. Worms recovered from different species of birds exhibited no constant differences in size, morphology or rate of development.

EGGS

The eggs (Fig. 3) are slightly elliptical with a terminal spine which is usually bent as a "J" and is rarely straight or absent. Measurements of more than 50 eggs from fresh preparations are 75–140 microns by 67–126 microns, the preponderance being in the greater size range. The spine averages about 6 microns. There were no appreciable differences in size or shape of Hawaiian schistosome eggs recovered

from different species of birds or from old or young worms. Occasionally very small eggs were found, but these were obviously necrotic and should not confound the diagnosis.

The eggs in the feces of laboratory birds contained active, mature miracidia. Laboratory experiments revealed that the miracidia in the capsules died as soon as the feces dried, or, if the feces remained moist, the miracidia died within an hour, apparently from the toxic effects of the feces. In the light of these observations and the greater hazards to which schistosome eggs are subject on the "bird islands," it seems most likely that, if the miracidia are to hatch, the feces containing them must fall directly into sea water.

MIRACIDIA

Twenty-five living, fully- or nearly-extended miracidia (Fig. 2), hatched in a flask such as described by McMullen and Beaver (1945), measured 183–225 microns by 33–40 microns. The epidermis is uniformly covered with long cilia except for the lateral papillary line about one-fourth of the body length from the anterior end and the almost imperceptible interstices of the epidermal cells. The anterior fourth of the body is occupied by 2 elongate, unicellular penetration glands and a broad, multicellular apical gland (primitive gut), the latter containing coarse, eosinophilic granules. Immediately caudad of these glands is a large sac-like organ, sometimes called the "posterior penetrating gland" (Faust, 1949) or "central nerve mass" (Ameel, Van der Woude, and Cort, 1953; H. F. Price, 1931; and others). The organ, however, lacks many of the characteristics of either a gland or a ganglion (central nerve mass). In the miracidia of the Hawaiian worm it lies free in the parenchyma except at either end where a few filamentous strands connect it with the anterior glands and the posterior propagatory cells. Histologically it is made up of an outer, single layer of cuboidal cells and an inner, acellular, fibrous, slightly eosinophilic matrix. The cells are small with scant cytoplasm and with nuclei containing rather thick chromosomes. Consequently, the organ stains a deep purple with hematoxylin.

There is evidence of the organ in some developing miracidia while the latter are still within the capsules in the tissues of the definitive host. However, it is no longer apparent within a short time after the miracidium has penetrated the intermediate host. To learn the fate of this organ a group of *Littorina pintado* were infected with miracidia, and, after increasing intervals of time, the mantles containing the young mother sporocysts were cut off, fixed and sectioned. The slides made from these tissues revealed that the organ persisted without perceptible change until 4 hours after miracidial penetration. Between 4 and 12 hours the cells became dissociated, and beyond 12 hours after penetration the site of the organ contained only a loose aggregation of cells with pycnotic nuclei. These cells dispersed and decreased in number until, 48 hours after penetration, they were no longer recognizable.

Because of the transient nature of the organ, its total histological dissimilarity to nerve tissue, and evidence of mitosis in its cellular components, it seems doubtful that it is a ganglion. Likewise, because of the small size, scant cytoplasm and pycnotic nuclei of its cells, and the fibrous nature of the interior matrix, it does not resemble a gland. Further studies are in progress to more conclusively determine its nature.

In the posterior half of the body are 6 to 12 propagatory cells more or less enmeshed in a medial, filamentous stroma. The excretory system consists of 2 pairs of flame cells, the anterior pair lying lateral to the "central nerve mass," and the posterior pair located medial and caudad of the nephridiopore. The flame cell capillaries unite about one-third of the body length from the posterior and then descend to the lateral nephridiopore which opens about one-fourth the body length from the posterior end.

The miracidia freed themselves from the capsules by rotating violently until the end opposite the spine gave way. In fresh, clear sea water (they died quickly in water contaminated with feces) the miracidia were constantly-active, elongate organisms that assumed a pyriform shape only when they collided with obstacles or were in an adverse environment. When miracidia were placed with snails they exhibited no special ability to find the mollusc. However, once accidental contact was made with the snail, penetration always took place along the lateral margin of the mantle. In this location the miracidia transformed into mother sporocysts.

SPOROCYSTS

The morphology and development of schistosome sporocysts have been described in considerable detail by H. F. Price (1931), Cort and Olivier (1943), Cort, Ameel and Olivier (1944), and Cort, Ameel and Van der Woude (1953). There do not seem to be any basic morphological differences between the sporocysts of *Austrobilharzia variglandis* occurring in Hawaii and those of *Schistosomatium douthitti* or *Trichobilharzia stagnicolae* as described by these workers.

Mother sporocysts removed from the mantle of *L. pintado* are translucent amorphous, sac-like organisms. Measurements of these sporocysts are not very significant because of their plasticity, but living ones measured 0.28–0.40 mm. by 0.12–0.14 mm. They attained their maximum size about 4 weeks after transformation from the miracidium and did not begin to decrease in size until after about 1 year. The first germ balls appeared at about 6 days, and their development into daughter sporocysts was rapid. The daughter sporocysts were first liberated between 30 and 40 days after miracidial penetration. They emerged from one end of the mother sporocyst through a protractile birth pore which had slightly lobulated margins. Mother sporocysts which were still liberating daughter sporocysts were recovered from the mantles of snails 1 year after infection. They seemed to have diminished in size and were opaque white due to an accumulation of oil droplets from fatty degeneration of most of the germ balls.

The daughter sporocysts of *A. variglandis* have already been described by Stunkard and Hinchliffe (1952). The measurements given for those of the Atlantic Coast are 0.654–1.38 mm. by 0.2–.392 mm. as compared to 0.63–0.84 mm. by 0.20–0.30 mm. for those of the Hawaiian worm. Little need be added to the existing description except that in the sporocysts from Hawaii an excretory system consisting of 4 pairs of flame cells was observed. The 4 flame cells on each side lie evenly spaced in the posterior half of the animal, with their capillaries uniting with the protonephridial duct near the anterior-most flame cell. The ducts then descend to the nephridiopore about one-fourth of the length of the sporocyst from the posterior end. It may be that 8 flame cells is not the total complement, but it was the greatest number that could be discerned in several favorable preparations.

After leaving the mother sporocysts, the daughter sporocysts migrate through

the tissues of the snail or possibly by way of the circulatory system, for they have on occasion been removed from the lumen of the snail's heart. Most of them become permanently entangled in the digestive gland of the snail, but in several heavy infections they were also found in the gonads. The daughter sporocysts first shed cercariae at about 4 months, and after 1 year were still shedding them without the slightest sign of abatement.

CERCARIAE

The cercariae of the Hawaiian *A. variglandis* are morphologically indistinguishable from those described and figured by Stunkard and Hinchliffe except for slight discrepancies in dimensions. The measurements in millimeters for body length, body width, tail stem length, furcal length, and acetabular diameter are 0.248, 0.091, 0.202, 0.108, and 0.028, respectively, for the Hawaiian form as compared with 0.237, 0.072, 0.228, 0.126, and 0.023 for the Atlantic Coast form. The cercariae from both the Atlantic Coast and Hawaii were first fixed in 10% hot formalin. Stunkard and Hinchliffe's figures represent averages of 10 specimens whereas those from Hawaii are averages of 50 specimens.

In Hawaii the *Littorina pintado* carrying infections of *A. variglandis* are found on water-covered sea benches of two "bird islands" (Moku Manu and Manana). In these areas about 1% of the snails are infected, with little or no seasonal fluctuation. In spite of the fact that *L. pintado* snails are very numerous in these areas and that flocks of sooty and noddy terns and golden plover (*Pluvialis dominica fulva* (Gmelin)) are frequently observed on the flats, not one of 40 terns and 12 golden plover collected on these benches were infected with *A. variglandis*. However, most people who have collected on these benches have, at one time or another, experienced cercarial dermatitis.

MODE OF INFECTION

In the laboratory natural emergence of cercariae from infected snails was always light even after the snails had been dried for several days before immersion. Consequently, all infections of laboratory animals were made by cracking snails and utilizing the "top swimming" cercariae. Liberated cercariae in fresh sea water gradually lost their ability to penetrate and generally died in about 18 to 24 hours when kept at room temperature. Refrigeration prolonged their life for periods up to 6 or 7 days, but the cercariae had then lost all their ability to penetrate. In 0.85% saline, at room temperature, they remained alive and normal in appearance for about 14 hours.

To preclude the possibility of naturally acquired helminthic infections in experimental definitive hosts, all domestic birds used were raised from newly-hatched stock and the terns, from downy young. Infection of the experimental birds was readily achieved by introducing cercariae into the eyes or cloacae or by immersing their feet in sea water containing cercariae. The cercariae usually penetrated in about 15 minutes. On only a few occasions did a noticeable dermatitis occur on the feet or tarsi of these birds. After cercarial penetration, the young worms reached the lungs in 3 days at which time they measured from 0.3 to 0.5 mm. in length.

Cercarial penetration in rats and man usually resulted in a mild dermatitis. Slides made from sectioned skin with cercarial lesions, taken from the abdomens

of 8 young rats, revealed that the penetrated cercariae lodged in both the dermis and subdermis and that the cercariae in the lesions were recognizable up to 4 days. The sections through the lesions showed a pronounced infiltration of eosinophils and lymphocytes to the immediate area. Two mice whose tails were exposed to cercariae, when killed and examined 4 and 5 days later, were found to have hemorrhagic areas and plasma-filled vesicles on their lungs. When the lungs were sectioned these areas were found to be heavily infiltrated with leucocytes, but no parasites were observed. It appeared that lung migration had occurred, but that the worms had already undergone lysis. Ability of this marine avian schistosome to reach the mammalian lungs appeared to be similar to that observed for fresh water avian schistosomes as reported by Olivier (1953).

The growth of *A. variglandis* in the lungs of terns or chickens was rapid, and by the sixth day, when most of the worms migrated to the mesenteric veins, they were already 1 to 2 mm. long. But after repeated infections it was observed that the young worms lingered in the lungs from 9 to 10 days and attained sizes up to 3 mm.

Observations based on autopsies of over 40 birds (terns, ducks and chickens) which had been subjected to either single or repeated infections indicated that migration of the worms to the mesenteric veins was direct and rapid. At the time of migration worms were usually found in either the pulmonary veins or mesenteric veins or in both but rarely elsewhere. On 2 occasions, however, worms probably in the act of migration were recovered from the chambers of the heart and the aortic arch. And, on 2 other occasions involving terns that had been repeatedly infected, 20 to 30 worms were recovered from the veins of the liver, and many eggs were present in the venules. The mesenteric veins of 1 tern contained an additional 20 worms but in the other bird no worms occurred in these vessels.

As soon as the worms reached the mesenteric veins the females entered the gynaecophoric canals of the males and 5 or 6 days thereafter were shedding eggs. Egg production by the female was always observed to be light.

Development of the sporocysts has already been mentioned but is here repeated, in part, for continuity. In the natural intermediate host (*Littorina pintado*), miracidia of *A. variglandis* were found to have penetrated the lateral margin of the mantle and there metamorphosed into mother sporocysts which remained in this location throughout the course of infection. Daughter sporocysts were first liberated between 30 and 40 days after miracidial penetration and migrated through the tissues of the snail to the digestive gland where they became enmeshed in the stroma of that organ. Cercariae were first shed about 4 months after infection of the snail, and such infections persisted for more than a year.

The natural definitive host (*Arenaria interpres interpres*) of *A. variglandis* in the Islands is a winter resident, arriving in September and usually departing in May. They are common throughout the Islands during that period, but only a few non-nesting individuals remain in the summer. Species of birds from the "bird islands," examined for schistosomes but found negative, are *Puffinus pacificus cuneatus* Salvin, *Pluvialis dominica fulva* (Gmelin), *Anous stolidus pileatus* Scopoli and *Sterna fuscata oahuensis* Bloxam.

In nature *L. pintado* are presumably infected by bird droppings containing the schistosome eggs which fall directly into the shallow water covering the snails. Three species of *Littorina* (*L. picta, L. undulata* and *L. pintado*) occur on the sea

benches of both Moku Manu and Manana Islands, but sporocysts have been found only in *L. pintado*. None of the several other species of operculate gastropods occurring in the same area [*Thais intermedia* (Diener), *T. harpa* (Conrad), *Drupa ricinus* (L.) and *Morula brunneolabrum* Dall] were ever found infected with larval schistosomes, nor were any of them capable of being experimentally infected. *Littorina scabra*, a fourth littorine species found in Hawaii, is a large form that spends most of its time above water in bay areas. Although examinations of over 5,000 *L. scabra* from various localities were negative for all types of trematode infection, snails of this species were infected experimentally with *A. variglandis* miracidia and yielded cercariae on cracking.

Two other trematodes utilize *L. pintado* on the "bird islands" as an intermediate host. Approximately 50% of these snails were infected with larval stages of *Parorchis acanthus* (Nicoll) and about 0.01% contained an undetermined, minute cercaria. Occasionally *L. pintado* snails were found with mixed infections of both *A. variglandis* and *P. acanthus*.

SUMMARY

The larval schistosome inhabiting the marine snail *Litorina pintado* Wood (previously reported from Hawaii) has now been identified as that of *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953. In the natural definitive host, *Arenaria interpres interpres* (L.), adult worms were recovered from the mesenteric veins.

Experimentally, chickens, ducks, noddy terns and sooty terns were succesfully infected with the cercariae of this schistsome. Although 4 species of littorine snails occur in Hawaii, only *L. pintado* was found to be a natural intermediate host. In the laboratory, however, *L. scabra* proved infectable with miracidia of *A. variglandis*.

A few morphological details of the adults and larvae of *A. variglandis*, not previously reported, are presented. In addition the mode of infection in both the intermediate and definitive hosts, as observed in the laboratory, are given.

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EXPLANATION OF FIGURES

FIG. 1. Male, *A. variglandis,* semi-diagrammatic composite drawing showing the general arrangement of the internal organs, especially the excretory system.

FIG. 2. Miracidium, *A. variglandis,* drawn from a photomicrograph of a living miracidium.

FIG. 3. Egg, *A. variglandis,* drawn from a photomicrograph of a living encapsulated miracidium isolated from feces of infected terns.

FIG. 4. Female, *A. variglandis,* semi-diagrammatic composite drawing, somewhat broader in proportion to its length in comparison with the natural appearance of a living or fixed specimen.

PLATE I

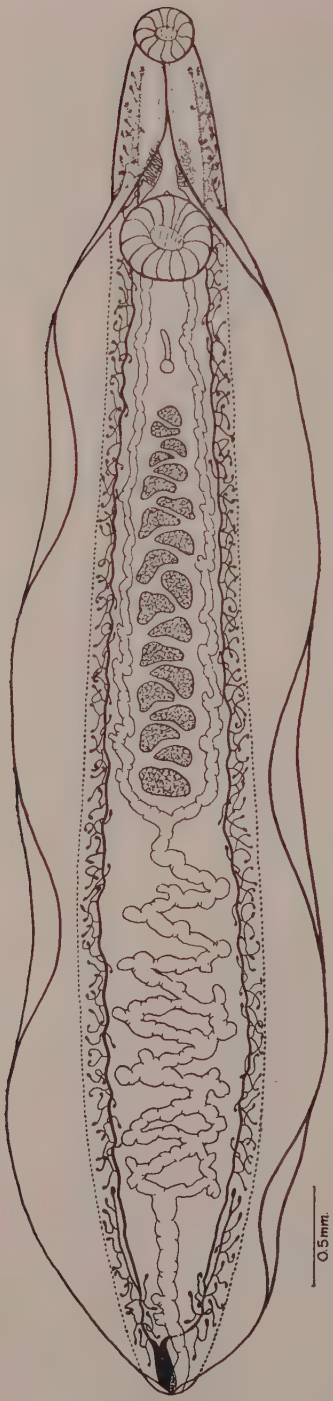

Fig. 1

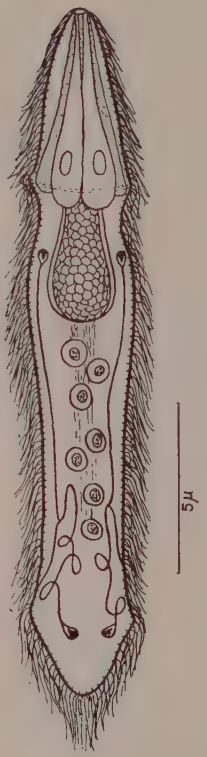

Fig. 2

Fig. 3

Fig. 4

THE EFFECTS OF THE PROTOZOAN PARASITE *EIMERIA TENELLA* ON THE ABILITY OF THE CHICKEN TO DO MUSCULAR WORK WHEN ITS MUSCLES ARE STIMULATED DIRECTLY AND INDIRECTLY*

LAURENCE LEVINE,
Department of Zoology
AND
CHESTER A. HERRICK
Departments of Zoology and Veterinary Science, University of Wisconsin, Madison

INTRODUCTION

The symptoms of cecal coccidiosis are many and varied and their severity is dependent upon numerous factors which include previous exposure to the parasite, the numbers of oocysts swallowed, and the age and genetic background of the host.

One of the early symptoms is the loss of appetite. Other symptoms such as loss of weight, anemia, reduced cloacal temperature and metabolic rate become progressively more marked reaching a climax with the hemorrhage into the cecal pouches on the fifth day after infection. At this time the host appears to avoid activity by standing listlessly in an isolated corner of the cage and is incapable of sustained action when disturbed. The host is apparently incapable of shivering but spontaneous erratic twitching of the muscles often occurs. The debilitation becomes increasingly severe until the host can no longer stand. Death appears to result from respiratory failure and is accompanied by only a slight struggle. If it is killed by decapitation or by breaking its neck, the vigorous convulsive contractions common to control chickens are markedly reduced.

Muscular weakness, therefore, seemed to be such an important factor in the syndrome of this and other parasitic diseases that the work which muscles of chickens infected with cecal coccidia could perform was measured and is herewith reported.

MATERIALS AND METHODS

The chickens used were Single-Comb White Leghorns furnished by the Department of Poultry Husbandry. They were raised free from infection in a building separate from the experimental laboratory until they were 10–12 weeks old. At this time, the individuals were paired for sex and weight. One member of each pair was infected orally with 150,000–200,000 sporulated *Eimeria tenella* oocysts, while the other served as its control. It should be mentioned that these animals were not particularly selected for susceptibility or resistance and that the determinations were made on the 5th and 6th days following infection.

The stimulating electrodes were made from materials commonly found in any laboratory. Bright platinum wires, 20 gauge, were masked by being housed in a trimmed and slit vaccine bottle cap (Figure 1). The platinum wires were forced through the walls of the cap in such a way that they lay perpendicular to the holes at

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the base of the slit, were 3.5 mm. apart, parallel, and in a plane slightly above that of the holes. These wires were then sealed at one end in glass capillaries and fine copper wires were soldered to the unsealed ends. Small rubber stoppers were cut to fit the housing and the bare wires outside this were insulated with a synthetic resin compound.

Approach to the sciatic nerve was gained, after etherization, by carefully incising both the biceps femoris and semitendinosis muscles in the pelvic region halfway between the anterior and posterior limits of the pelvic girdle. The sciatic nerve and all the auxiliary branches leading into the ischiadic foramen thus exposed were

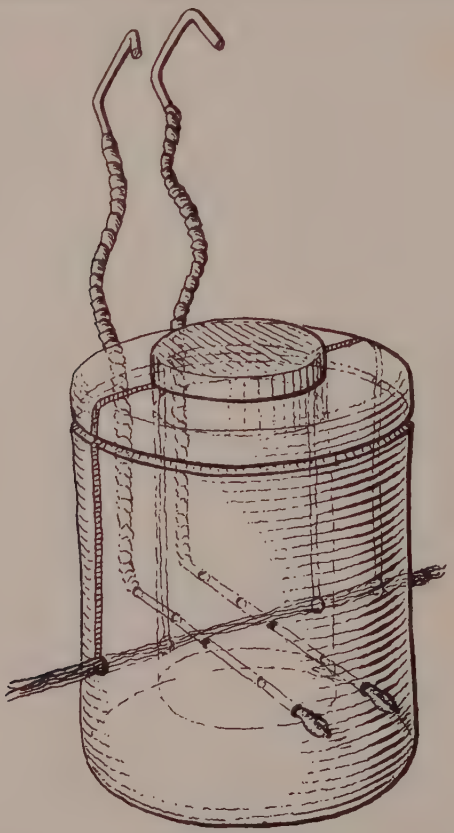

FIG. 1. Trimmed and slit vaccine bottle cap masking the platinum stimulating electrodes.

severed as far centrally as possible. The wound was closed and the procedure repeated on the opposite side. The sciatic nerve was prepared to receive the stimulator by exposing it distally to the peroneal branch which was then cut. The intact tibial branch was then lifted and gently slipped into the stimulating assembly via the two opposing slits so that the nerve lay over the platinum electrodes. The entire assembly was then capped with a rubber stopper and bound with a suture in the appropriate groove on the electrode housing (Figure 1). The wound was sutured and a thin coat of celloidin applied to the area.

The gastrocnemius muscle was prepared for recording by turning the animal

ventrally and exposing the tendon of Achilles by making an incision 2–4 cm. long, slightly below the tibiotarsal joint. After a light hook was engaged in the cartilaginous portion of this tendon, it was cut, lifted, and freed of fascia cephalad. The tendons from the anterior and tibial heads of the gastrocnemius were severed at their junction with the tendon of Achilles. The wound was sutured and a wet dressing applied. The part of the gastrocnemius used, therefore, was that which originates from the external tuberosity of the femur. After this procedure was repeated on the opposite leg, the bird was placed in a thermostatically controlled cage (about 30° C.) where it remained for approximately 2 hours. At the end of

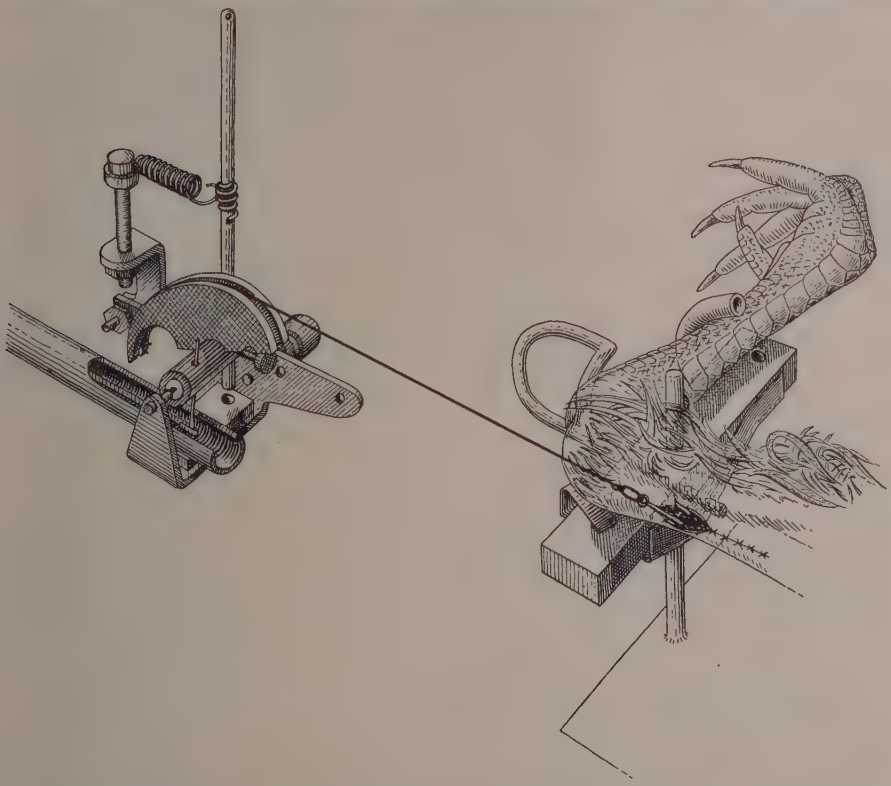

FIG. 2. Mechanism for immobilizing leg of chicken and modified muscle lever used to measure work output.

this period, the animal had completely recovered from the anesthetic and appeared to be comfortable.

The animal was then placed in a position to record. The leg was held rigidly by means of an arrangement (Figure 2) which did not seem to interfere with circulation. The muscle was attached to the recording system by means of its hook and a fine steel wire which contained an insulating segment. Appropriate electrical contact was then made between the stimulus generator and the copper wires of the masked platinum electrodes which extended out of the closed and sealed wound. The stimulus was generated in a Arthur Thomas Model No. 751 stimulator at a frequency of 3 pulses per second and a duration of 0.05 milliseconds. The intensity

of stimulation was supramaximal and the polarity adjusted so that the current was anodal to the nerve and ascending in all determinations. The stimulations were continued for a period of at least 3 minutes during which time the muscle contracted against a spring in the lever system which was calibrated before and after each set of determinations. The amplitude of contraction produced by the gastrocnemius under the above conditions was converted to work in gram centimeters with the aid of calibration data and the appropriate corrections applied for the magnification of the lever system. The areas beneath the curves were calculated with the aid of a planimeter and this was used to determine the total amounts of work.

After being disconnected from the electrical and recording apparatus, the animal was turned over, covered, and heat applied from an overhead heating lamp for a period of at least one hour to prevent chilling. Direct stimulation was initiated by inserting 24 gauge platinum wires soldered to fine copper wires into the muscle, through the skin, after its tendon was attached to the recording system. In these tests the duration of stimulus was increased to 0.5 milliseconds, but the frequency remained the same. The stimulus intensity was maximal. That the time interval between direct and indirect stimulation was of sufficient length to rule out the possibility of interference of one with the other was ascertained by previous experiments. Furthermore, cloacal temperatures taken before recording periods for each of the opposite sides revealed no appreciable change.

After the phase of direct stimulation was completed, the animal was detached from the apparatus, its gastrocnemii were rapidly excised and weighed on an analytical balance.

Previous experiments showed that it was not feasible to use ether or nembutal during the period of recording, for various inconsistencies of performance were found to arise from the difficulty in producing the same level of narcosis in all animals and furthermore, there was a possiblity that anesthetics may affect the neuromuscular apparatus (Heinbecker and Bartley, 1940). With ether anesthesia the animal would consistently awaken and disrupt the apparatus in attempts at flight. The problem was even more pronounced in the case of infected chickens for they showed extreme susceptibility to nembutal. The most satisfactory results were obtained by giving ether anesthesia and transecting both sciatic nerves as described above. Animals so treated recovered from the anesthetic promptly and seemed to be comfortable during the recording process.

RESULTS AND DISCUSSION

The curves which represent the average performance of the controls stimulated indirectly (through the nerve) are shown in Figure 3. The muscles of these animals exhibited an increased ability to do work (treppe) reaching a maximum of 233 gm. cm. per contraction at 90 seconds. Fatigue ensued gradually beyond this point and work fell off to 182 gm. cm. per contraction at the end of 180 seconds when the experiment was terminated. The infected animals, however, were capable of doing essentially the same amount of work per contraction as the controls for 30 seconds when they reached their maximum which was less than the work output of the controls at the end of the test period. A calculation of the total amount of work (Table 1) showed that the infected chickens fatigued after doing ¼ the work that the controls had done before they fatigued. Similarly, by the end of the determination, the controls did 1.7 times more work than the infected animals.

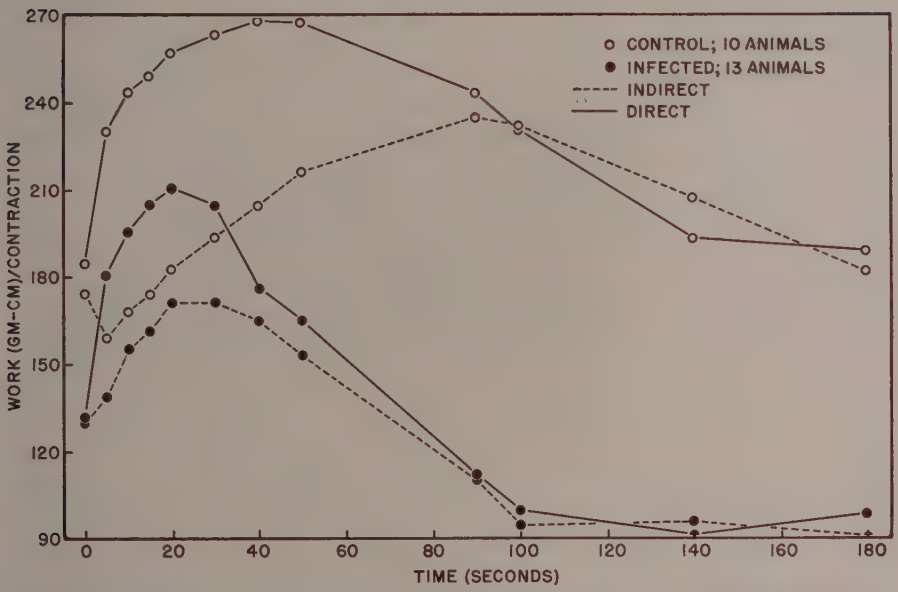

FIG. 3. Average work output (gm. cm./contraction) of direct and indirectly stimulated gastrocnemii of chickens infected with cecal coccidia and their controls.

Significance of differences between infected and control chickens: When stimulated indirectly $P > 0.05$ from 0–20 secs. $P < 0.05$ at 30 and 40 secs. $P < 0.01$ from 50–180 secs.

When stimulated directly $P = 0.02$ at 0 and 10 secs. $P < 0.05$ at 5, $P = 0.05$ at 15 secs. and 30 secs. $P < 0.01$ from 40–180 secs.

When the muscles of the opposite legs of the animals mentioned above were stimulated directly the controls reached a maximum of 268 gm. cm. per contraction in 40 seconds and were doing 190 at the end of the determination. The average maximum work per contraction of the infected birds was 69% that of the controls ($P < 0.05$) and fatigue occurred in one half the time. The controls did 3 times as much work as the infected chickens before they fatigued and 1.7 times as much during the entire test period.

A comparison of the work output of the directly and indirectly stimulated muscles showed that the maximum response of the controls to direct stimulation was significantly higher ($P < 0.01$) than that of indirect stimulation and occurred much earlier. When the muscles of infected animals were stimulated directly they did significantly more work per contraction than did the indirectly stimulated muscles but the time required to reach the peak of work output was the same in both cases. The responses of the muscles of both control and infected chickens to direct stimu-

TABLE 1.—*Tabulation of work done by the gastrocnemii of infected and control chickens shown in figures 3 and 4*

	Indirect stimulation		Direct stimulation	
	Infected	Control	Infected	Control
Fig. 3				
Before fatigue	4,734*	18,546	3,810	11,988
Total	21,768	37,326	23,754	41,550
Fig. 4				
Before fatigue	1,302	3,990	1,008	2,334
Total	5,955	8,143	6,292	8,332

* All amounts = gram – centimeter – seconds.

lation were greater than those resulting from indirect stimulation. This difference in response to direct over indirect stimulation was not only greater in the controls but was maintained for twice as long as it was in the infected animals.

The muscles of infected and control chickens were weighed in the manner previously described. The weights of the gastrocnemii of infected animals averaged 3.71 gms. while those of the controls averaged 4.82 gms. The percentage dry weight of wet weight did not change appreciably during the course of the infection, indicating that the loss of weight of these muscles was not caused by a loss of tissue water alone but by a loss of tissue components in the same proportion as water. With this in mind, the ability of the muscles to do work was compared on a per gram basis (Figure 4) to determine whether or not the decreased maximum ampli-

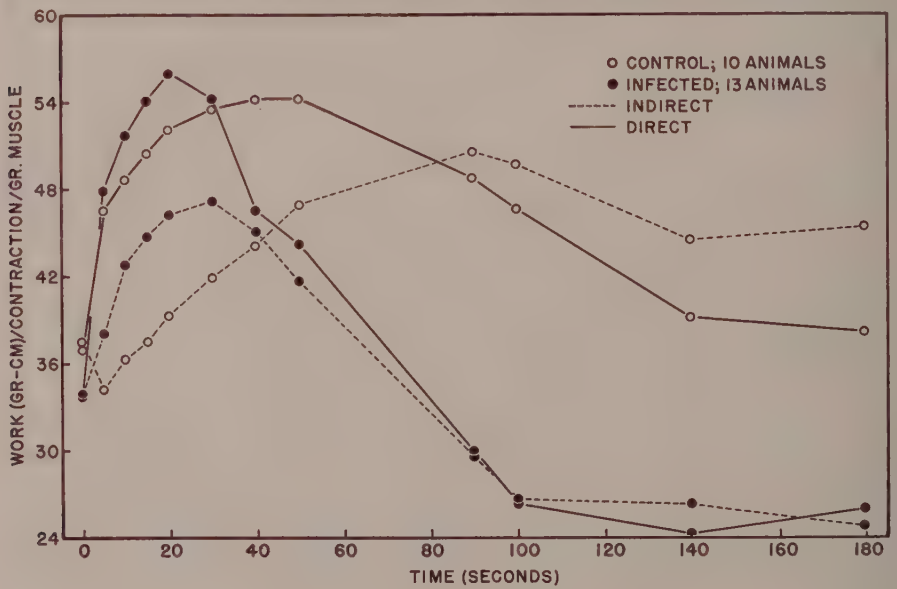

FIG. 4. Average work output (gm. cm./contraction/gm. muscle) of direct and indirectly stimulated gastrocnemii of chickens infected with cecal coccidia and their controls.

Significance of differences between infected and control chickens: When stimulated indirectly $P > 0.05$ from 0–50 secs. $P = 0.02$ at 90 secs. $P < 0.01$ from 100–180 secs.

When stimulated directly $P > 0.05$ from 0–40 secs. $P < 0.05$ at 50 secs. $P = 0.02$ at 90 and 140 secs. $P < 0.01$ at 100 and 180 secs.

tude of work per contraction was related to the emaciation of the muscle. A statistical analysis of these data showed that the muscles of the infected animals could do as much work per gram per contraction as the controls, but due to early fatigue they could do only 73.1% as much work in the 3 minute test period.

Although our data adequately support the idea that cecal coccidiosis significantly reduces the ability of the muscles of chickens to do work, no adequate explanation can be offered regarding the mechanism involved. Such factors as the inhibition of phosphorylation (Daugherty and Herrick, 1952), reduced glycogen (Waxler, 1941; Pratt, 1941) and anemia (Natt, 1951) may play a part in the causation of this phenomenon. Experiments designed to determine the possible involvement of the nervous system are now in progress.

Experiments similar to those reported above are now being carried out on Plymouth Rock chickens since heavier breeds usually suffer less from cecal coccidiosis. Although the general effects of the coccidia are similar many differences are evident. The details of these studies will be reported later.

As a matter of interest it should be noted that the muscles of the right legs were heavier than those of the left in both experimentals and controls. This difference in weight, however, was significantly higher ($P < 0.01$) only in the controls.

SUMMARY

1. When the gastrocnemii of chickens infected with cecal coccidiosis were stimulated directly or indirectly they did only 58% of the work done by the controls.

2. Early and pronounced fatigue was characteristic of the muscles of the infected chickens.

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THE OXIDATIVE METABOLISM OF *TRYPANOSOMA LEWISI* FROM SALICYLATE TREATED INFECTED WHITE RATS

JEANNE B. ZWISLER[1] AND MICHAEL G. LYSENKO[2]

INTRODUCTION

Taliaferro (1932, 1948) described the course of infection, the changes in the character of the parasitic population during the course of the infection, and the subsequent immunity of the rat to reinfection with *Trypanosoma lewisi*. The immune response in white rats was ascribed by Taliaferro (1948) to the appearance of 2 antibodies, ablastin and the trypanocidal factor. Becker and Gallagher (1947) found that the reproductive phase of the *T. lewisi* infection was prolonged and the parasitemia increased by the administration of sodium salicylate to the infected animal. Saul and Becker (1949) suggested that the trypanocidal antibody remained unaffected in salicylate treatment and that only ablastic activity was affected. Lysenko (1951) experimenting with sodium salicylate and ablastic plasma from white rats infected with *T. lewisi* presented evidence against any combination of salicylate with the ablastic antibody either *in vivo* or *in vitro*. It was suggested by Lysenko that salicylate treatment in some way prevented the formation of ablastin. The effect of ablastin upon the oxidative metabolism of *T. lewisi* from non-salicylate

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[1] Part of a thesis submitted in fulfillment of the requirements for the Ph.D. degree at the University of Wisconsin, Madison. Present address at the Department of Biochemistry, University of Texas, Medical Branch, Galveston.

[2] Department of Zoology and Medical Microbiology, University of Wisconsin, Madison.

treated animals was discussed by Moulder (1948b) in which it was shown that normal dividing populations (4–5 day) used less oxygen and more glucose than normal (9–10 day) trypanosome populations. This investigation proposed to determine whether similar relationships prevailed in salicylate-treated animals with older but still multiplying populations.

MATERIALS AND METHODS

A strain of *Trypanosoma lewisi* isolated by Becker at Iowa State College in 1943 was inoculated intraperitoneally into white male rats (Wistar strain) ranging in weight from 200 to 300 grams. Salicylate treatment followed the method of Saul and Becker (1949). Preparation of the parasites for Warburg analysis was undertaken according to Moulder (1948a). Population studies followed Becker and Gallagher (1947). The parasites were suspended in the Warburg flasks in the following modified medium of Ryley (1951): 0.1 M sodium chloride, 0.007 M potassium chloride, 0.002 M magnesium sulfate, 0.016 M sodium bicarbonate, 0.0184 M potassium dihydrogen phosphate and 0.0812 M potassium monohydrogen phosphate. In all determinations requiring glucose 0.1 ml. of a stock solution of glucose containing 3.6 gm. per 100 ml. of distilled water was used. Glucose analysis followed a modified Folin-Wu method (Hawk, Oser and Summerson, 1947). Temperature was maintained at 37° C. The gas phase was either air or 95% nitrogen plus 5% carbon dioxide. Evolution of carbon dioxide as a measure of acid production followed the method outlined by Umbreit (Umbreit, Burris and Stauffer, 1949). Each flask contained 2×10^8 trypanosomes suspended in 3.2 ml. fluid. Determinations were one hour in length. Statistical analyses were carried out according to Snedecor (1950).

EXPERIMENTAL RESULTS AND DISCUSSION

In the following series of experiments the trypanosomes were of 4 population types (4N, 9N, 4S, 9S) depending upon the age of the infection and the treatment to which the host animal was subjected. The 4N and 9N series consisted of trypanosomes obtained from the 4 and 9 day infections of untreated rats. The corresponding 4S and 9S infections were obtained from salicylate-treated infected animals.

In both treated and untreated infected animals peak numbers of division forms were obtained by the fourth day after infection. The average percentage of division forms in the 4S series was 50%, while that of the 4N series averaged 43%. Reproducing forms in the untreated animals rapidly declined in numbers until they completely disappeared by the ninth day. The 9S series consisted of 22% reproducing forms.

Table 1 indicates the results of a series of experiments to determine the oxygen uptake of trypanosomes obtained from untreated infected and salicylate-treated infected animals. It was found that in the 4N and 9N infections as the age of the infection increased the amount of oxygen utilized also increased. A 4N infection consisting of 43% division forms was found to have a mean oxygen uptake of 125.5 cu. mm. as compared with the 9N population in which no division forms existed having a mean oxygen uptake of 151.3 cu. mm. These results were consistent with the investigations of Moulder (1948b). Moulder also proposed that the shift in oxidative metabolism was correlated with the appearance of the antibody, ablastin.

The oxygen uptake of the 4S and 9S populations showed no significant difference in the oxygen utilization, the average uptake being 92.4 cu. mm. in the 4S and 102.2 cu. mm. in the 9S populations. Lysenko, on the basis of decrease in the total nitrogen and electrophoretic analysis of serum proteins (1951), showed that salicylate in the rat suppressed ablastin formation. The similarity of the oxidative metabolism of the 4S and 9S groups of trypanosomes presented further evidence in support of this assumption.

The difference in oxygen uptake between the 4N and 4S groups may in this case be an indication of early appearance of ablastin. Becker, Manresa and Johnson (1943) believed that the host exhibited a high degree of resistance for the particular strain of *T. lewisi* used in these experiments as evidenced by lower maximum numbers of trypanosomes and shorter duration of infection than was seen with the strains used by Taliaferro (1932) and Moulder (1948a, b).

Aerobic glucose utilization by the 4N and 9N populations averaged 0.33 mg. per hour and 0.36 mg. per hour respectively. Trypanosomes from salicylate treated animals averaged a somewhat higher utilization of glucose per hour ranging from 0.38 mg. in the 4S group to 0.43 mg. in the 9S group. Statistical analysis indicated no significant difference.

TABLE 1.—*Comparison of the O_2, CO_2, exchange of aerobic and anaerobic glucose metabolizing* Trypanosoma lewisi *from salicylate-treated and untreated infected white male rats*

Population	Number of determinations		Average cu. mm. gas exchange per 15 min. period											
			1		2		3		4		Total		± ***	
	O_2*	CO_2**	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
4N	33	8	31.9	24.3	31.2	26.9	32.4	23.3	30.0	20.1	125.5	94.6	5.8	3.3
4S	21	3	23.4	21.9	19.7	32.0	26.8	15.0	22.5	23.6	92.4	92.5	7.2	9.8
9N	96	33	40.9	21.1	37.4	17.3	36.4	16.9	36.6	17.5	151.3	72.8	3.6	4.7
9S	50	11	26.4	23.9	27.1	18.2	25.3	21.3	23.4	17.2	102.2	80.6	3.4	6.8

*O_2—aerobic
**CO_2—anaerobic
***±—Standard error based on deviation of individual hour totals from the mean of the hour totals.

Repetition of the above series of experiments under anaerobic conditions (Table 1) showed little statistical variation in the amount of carbon dioxide produced. The mean total values ranged from 72.8 cu. mm. in the 9N to 94.6 cu. mm. in the 4N groups. The 4S and 9S populations averaged 92.5 cu. mm. and 80.6 cu. mm. respectively.

Anaerobic glucose utilization showed a somewhat higher value than that obtained aerobically. The 4N and 9N groups used 0.45 mg. glucose per hour while the 4S populations averaged 0.47 mg.

It was found that aerobic experiments with trypanosomes from salicylate and non-salicylate treated hosts presented a striking difference in oxygen utilization. In the 4S and 9S populations the amount of oxygen used was approximately the same. With the appearance of ablastin the oxygen rate increased approximately 50% as shown in the comparisons made between the 9N and 9S populations, a possible indication of greater activity on the part of the oxidative enzyme systems. An idea first suggested by Taliaferro (1948) and further supported in these investigations was that the normal route of metabolism of a reproducing population of *T. lewisi* was less efficient in its metabolism than that of the adult or near adult populations

which were subjected to the additional stress factor of ablastin. The increased efficiency of glucose utilization as shown by the increased oxidative activity may well be necessary for maintenance of an adequate cellular balance. At this time there is no evidence to indicate the oxidative enzyme system which may become active in the metabolism of the adult trypanosome populations.

The *in vitro* effect of sodium salicylate upon metabolism was also investigated. To each experimental Warburg flask containing 9N trypanosomes, 0.2 ml. of a 0.1 M solution of sodium salicylate in distilled water was added. No significant difference was noted in the oxygen uptake between 4 control flasks which used an average of 124.1 cu. mm. oxygen and 5 flasks containing salicylate which used an average of 118.8 cu. mm. oxygen. These results indicated that salicylate as such had little if any effect upon trypanosomes not previously exposed to salicylate treatment in the host animal.

An attempt was made to demonstrate an *in vitro* effect of immune serum upon oxygen uptake. To each flask 1.0 ml. of immune serum from the 9N infection was added and the serum plus trypanosomes diluted to standard volume (3.2 ml.). It was expected that the oxygen uptake of the 15 flasks containing 4N and 9N serum would approximate the oxygen uptake of the 12 flasks containing 9N in 9N serum. However, there was no indication of an *in vitro* effect, the oxygen uptake of the 4N infection in serum averaged 88.9 cu. mm. oxygen while that of the 9N trypanosomes in similar serum averaged 132.1 cu. mm. oxygen. One phenomenon was noted. Upon addition of the 4N trypanosomes to the 9N serum agglutination occurred, a manifestation of the trypanocidal antibody. The greater susceptibility of the reproducing trypanosomes to agglutination was noted by Augustine (1943). The agglutination and subsequent decreased motility of the 4N trypanosomes in 9N serum in this case may account for deviation from the expected results.

SUMMARY AND CONCLUSIONS

Experiments with *T. lewisi* infections obtained from salicylate-treated and untreated infected rats showed that the typical lower oxygen uptake of the reproducing populations was maintained in trypanosomes obtained from salicylate-treated infected rats. There was no evidence found to support any *in vitro* effect of sodium salicylate upon trypanosomes not previously exposed to salicylate in the rat.

Negative results were obtained in the series of experiments attempting to demonstrate an *in vitro* effect of immune serum upon oxygen uptake by the addition of immune serum to trypanosomes respiring in Warburg flasks.

Under anaerobic conditions the amount of carbon dioxide produced by infections from salicylate-treated and untreated infected rats was essentially the same.

Findings on the change in oxygen uptake in the normal infections corroborated those of Moulder (1948b). However, a corresponding change in glucose consumption could not be shown.

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TWO NEW CESTODES OF THE GENERA *IDIOGENES* KRABBE, 1868 AND *CHOANOTAENIA* RAILLIET, 1896

P. N. Chatterji
Zoology Department, Ravenshaw College, Cuttack, India

Krabbe (1869) proposed a new genus, *Idiogenes,* for *Taenia otidis* Werner. For this genus a new sub-family, Idiogeninae Fuhrmann, 1907, under the family Davaineidae Fuhrmann, 1907, was created. Kholodkowskii (1905), Fuhrmann (1909, 1932), Meggitt (1927 and 1933), Joyeux, Baer and Martin (1936), and Schultz (1939) each contributed a new species to this genus. Only one species, viz. *I. purtiva* Meggitt, 1933, thus far is known from India. Below is added a second species from India.

Long after the erection of the genus *Choanotaenia* Railliet (1896) there was controversy regarding the validity of the genus, but finally various helminthologists have accepted this genus for those species in which the uterus breaks up into egg capsules. To this genus I am adding a new species, described in the latter part of this paper.

Idiogenes butasteri n sp.
(Figures 1, 2, 3.)

About 10 specimens of this cestode were collected from the intestine of 1 of 5 kites, *Butaster tisa,* examined.

Description: Strobila, about 5 cm. long, containing few more than 200 proglottides. Scolex (Fig. 1) well-developed, broader than long, 0.069–0.126 mm. long and 0.112–0.21 mm. broad. Unsegmented portion of body 0.165–0.49 mm. long and 0.069–0.112 mm. broad, just behind scolex modified into pseudoscolex. Rostellum well-developed, 0.046–0.092 mm. long and 0.049–0.135 mm. broad, protruding from rostellar sheath, and armed with many small hammer-shaped spines arranged in 2 alternating rows. Suckers 4, almost spherical, 0.043–0.049 mm. long and 0.039–0.049 mm. broad, armed with many small hammer-shaped spines arranged in 3 or 4 overlapping rows. Genital primordia beginning about 70th to 100th segment and becoming matured at about 88th to 130th segment from the scolex. Matured proglottides (Fig. 2) trapezoidal, with proximal end narrower and distal end broader, 0.266–0.49 mm. long and 0.294–0.532 mm. in maximum

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breadth. Gravid segments (Fig. 3) much elongated, caducous, 1.25–2.52 mm. long and 0.49–0.63 mm. broad. Genital pores marginal, unilateral, and in anterior half of proglottides.

Testes follicular, 8 in number, post-ovarian, overlapping one another, follicles 0.053–0.066 mm. long by 0.049–0.066 mm. broad. Cirrus sac large, extending nearly to middle of segment, 0.214–0.264 mm. long and 0.082–0.099 mm. broad. Cirrus (Fig. 2) very large when protruded and armed for a considerable distance with numerous minute backwardly directed spines. Vas deferens and vesicula seminalis not properly differentiated, but small duct, the vesicula seminalis externa, full of spermatozoa, seen.

Ovary pretesticular, median, bilobed. Ovarian lobes slightly elongated or spheroidal, joined by narrow isthmus. Poral lobe slightly larger, 0.043–0.089 mm. long and 0.049–0.112 mm. broad. Aporal lobe 0.033–0.099 mm. long and 0.056–0.099 mm. broad. Terminal portion of vagina lying posterior to cirrus sac, and opening into common genital atrium. Vagina armed for considerable distance with numerous small backwardly directed spines. Receptaculum seminis not well seen. Uterus (Fig. 3) well-developed, forming an inverted "U" attached to well developed paruterine organ. In caducous segments uterus more or less sac-shaped and full of hexacanth embryos. Paruterine organ in gravid segments well-developed, 0.35–0.798 mm. long and 0.168–0.182 mm. broad. Vitellarium compact, spherical, 0.033–0.059 mm. long and 0.04–0.063 mm. broad, lying just behind ovary and shifted slightly towards poral end.

Host: Butaster tisa.

Habitat: Intestine.

Locality: Allahabad, Province Uttar Pradesh, India.

Type material: In author's collection.

DISCUSSION

I. butasteri n. sp. comes close to *I. buteonis* Schultz, 1939 in having its suckers armed with spines, in general shape of the proglottides, in its unilateral genital pores, armed cirrus and vagina and having as host a bird of the order Accipeters. But the new species can easily be distinguished by the considerably larger size of its strobila, by the presence of a pseudoscolex, fewer testes, 8 in *I. butasteri* and 12–18 in *I. buteonis,* by its large and protruding cirrus and in having a very inconspicuous vas deferens and a small uncoiled vesicula seminalis.

I. butasteri differs from *I. purtiva* Meggitt, 1933 in general shape of the proglottides, number and position of the testicular follicles (8, all posterior to the female genitalia in *I. butasteri* and 20–28 posterior and lateral to the female genitalia in *I. purtiva*), in size and shape of the ovary (bilobed in *butasteri,* unlobed in *purtiva*), in having unilateral genital openings in contrast to irregularly alternating openings in *purtiva,* and its suckers armed with spines. Besides having its suckers armed with spines, *I. butasteri* can be differentiated from the remaining species of the genus in many other characters, such as the position of the genital pore, number and size of the testes, size of the cirrus sac and cirrus, and coiled or simple vagina.

Choanotaenia bhattacharai n. sp.

Figures 4, 5, 6.

Six specimens of this cestode were collected from the small intestine of a small water bird, *Querquedula querquedula.* One of 2 hosts examined were infected. This species is named after my professor, Dr. D. R. Bhattacharya, ex-vice chancellor of the Univerity of Allahabad.

Description: Worms consisting of about 30–40 segments each, total length about 1.5–3 cm. by 0.896–1.036 mm. in maximum breadth in hindermost gravid segments. Scolex (Fig. 4) well-developed, much broader than long, 0.252–0.336 mm. long by 0.42–0.462 mm. broad. Rostellum well-developed, 0.252–0.28 mm. long and 0.098–0.125 mm. broad. Rostellar sheath also well-developed, extending beyond posterior margin of suckers. Rostellum armed with about 14 well-developed rostellar hooks, arranged in single row, each hook (Fig. 5) measuring 0.059–0.066 mm. long and 0.010–0.013 mm. in maximum breadth. Suckers 4, unarmed, well-developed, almost spherical, 0.182–0.196 mm. long by 0.154–0.168 mm. broad. Unsegmented neck region absent. Genital primordia beginning about 12th to 16th segment, and sex glands well matured at 17th to 20th segment. Anterior segments very small, narrow, much broader than long. Mature segments (Fig. 6) broader than long, 0.28–0.56 mm. long by 0.522–0.812 mm. broad. Gravid

proglottides also broader than long, 0.602–0.854 mm. long by 0.896–1.036 mm. broad. Genital pores marginal, regularly alternating, and situated near anterior corner of proglottides.

Testes follicular, numbering about 16–20, postovarian, overlapping one another. Each follicle nearly spherical, 0.053–0.084 mm. long by 0.056–0.098 mm. broad. Cirrus sac large, thin-walled, extending nearly to middle of segment. Cirrus small, unarmed, not seen protruded. Vesicula seminalis externa represented by loosely coiled vas deferens.

Ovary well-developed, pretesticular, nearly median, consisting of 2 wings joined by narrow isthmus. Each ovarian lobe transversely elongated and slightly indented. Aporal lobe 0.036–0.099 mm. long by 0.099–0.165 mm. broad and poral lobe 0.026–0.082 mm. long by 0.082–0.165 mm. broad. Vagina passing posterior to cirrus sac and opening separately a little behind opening of cirrus sac. Receptaculum seminis very small, placed just behind ovarian isthmus. Uterus in mature segments not distinguished properly, but in gravid segments broken up into many capsules distributed throughout breadth of segment, each containing single onchospheres. Vitellaria compact, well-developed, nearly spherical, 0.039–0.079 mm. long and 0.046–0.098 mm. broad, lying just behind ovarian isthmus, but shifted slightly towards aporal side.

Host: Querquedula querquedula.

Habitat: Small intestine.

Locality: Gorukhpur, Province Uttar Pradesh, India.

Type material: in author's collection.

DISCUSSION

Ch. bhattacharai n. sp. comes very close to *Ch. microsoma* Southwell, 1922 in general topography, having the same number of proglottides, which are broader than long, the same number of testes, absence of neck region, and egg capsules each containing a single onchosphere; but *Ch. microsoma* can be very easily differentiated from the new species by the different shape and size of the scolex, by the shape, size and much lobed condition of the ovary, by the cirrus which is peculiarly armed with spines set at right angles to its length, and immediately median to its tip the cirrus is armed with a number of hooks of different shape. The cirrus is simple and unarmed in the new species.

Ch. bhattacharai n. sp. resembles *Ch. chandleri* (Moghe, 1925) Fuhrmann, 1932 with respect to general topography, segments broader than long, bilobed ovary and egg capsules each containing a single onchosphere. *Ch. bhattacharai* can very easily be differentiated from *chandleri* by its fewer proglottides, 30–40 in contrast to about 135 in *chandleri,* also in the absence of a neck region, fewer and smaller rostellar hooks, in having the genital pore regularly alternating in contrast to the irregularly alternating genital pores in *chandleri,* in having a non-protrusible cirrus in contrast to a protruded cirrus in *chandleri.*

Ch. taylori Johnston, 1912 resembles the new species in general topography, the genital pore being regularly alternating and in having the same number of testes; but it differs in the shape and size of the strobila, shape and size of the vitellaria, in the presence of spines on the cirrus (cirrus aspinose in the new species), and much smaller scolex (0.13 mm. in diameter in *taylori* as compared with 0.252–0.336 mm. by 0.42–0.462 mm. in the new species).

From other species of *Choanotaenia* the new species differs in many important characters, such as the number, shape and size of rostellar hooks, number of testes follicles, character of alternation of genital pores, shape and size of the ovary, differences in number, shape and size of the proglottides, and in number of onchospheres contained in egg capsules.

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Explanation of Plate

Abbreviations used: C, cirrus; Cs, Cirrus sac; Ex Cl, excretory canal; Em, embryo; Gp, genital pore; Ov, ovary; PO, paruterine organ; R, rostellum; R Sp, Rostellar spine; S, sucker; Test, testis; Ut, uterus; Vit, vitellaria; Ves Sem, vesicula seminalis.

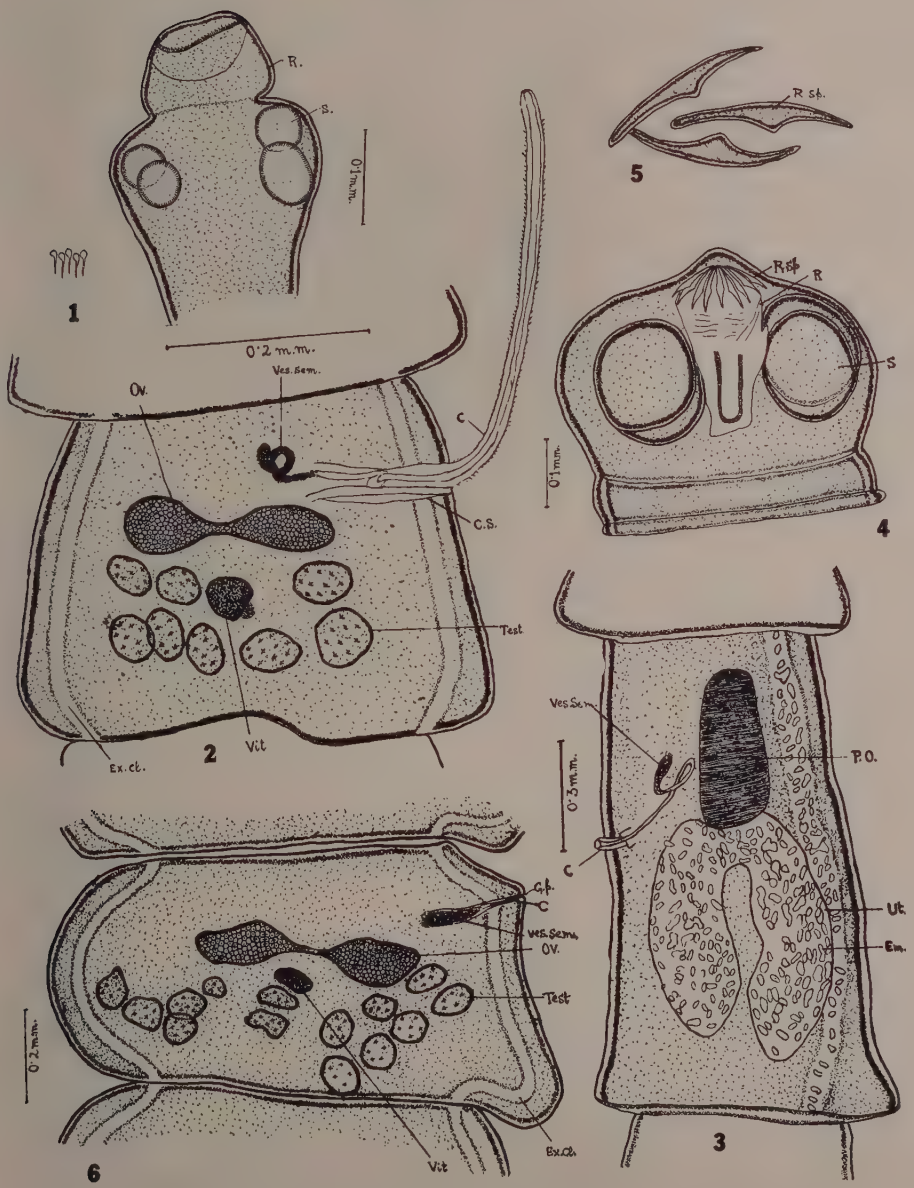
Idiogenes butasteri n. sp,

1. Scolex and spines.
2. Matured proglottis.
3. Gravid proglottis.

Choanotaenia bhattacharai n. sp.

4. Scolex.
5. Rostellar hooks.
6. Matured proglottis.

PLATE I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STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXI. TAXONOMY, MORPHOLOGICAL VARIATION, AND ECOLOGY OF *DIPHYLLOBOTHRIUM URSI* N. SP. PROVIS. ON KODIAK ISLAND

ROBERT RAUSCH*

According to Eguchi (1934), two species of salmon (*Oncorhynchus*) serve as a source of human infection by a cesode indentified as *Diphyllobothrium latum* (Linnaeus, 1758), in Japan. The possible role of these fishes in the transmission of cestodes to man and other animals has not been investigated in North America, nor, apparently, on the Eurasian mainland. However, Ward (1930) reported unidentified *Diphyllobothrium*-like plerocercoids from Alaskan salmon; Simms and Shaw (1931) collected plerocercoids identified as *D. cordiceps* (Leidy, 1871) from *Oncorhynchus kisutch* (Walbaum) from lakes in Oregon; and Wardle (1932) reported plerocercoids of *Diphyllobothrium* spp. from *O. kisutch* and *O. nerka* (Walbaum) in western Canada.

Cestodes of the genus *Diphyllobothrium* Cobbold, 1858, are commonly harbored by bears on Kodiak Island, in the Gulf of Alaska. During the months of summer and early fall, these mammals feed largely upon salmon, which spawn in great numbers in the streams of the island. Since fishes of other species are rarely obtainable, with the exception of a herring, *Clupea pallasii* Valenciennes, which spawns along the coast in early spring, the circumstances strongly suggest that the bears are infected through ingesting plerocercoid larvae in salmon.

Five species of *Oncorhynchus* occur in the waters of Kodiak Island, and all are important as human food. Aboriginal methods of preparing fishes for human consumption are such that cestode larvae might remain viable for some time. Localization of plerocercoids in parts of the fish used for food would provide opportunity for human infection.

An investigation of the ecology and taxonomy of the species of *Diphyllobothrium* occurring on Kodiak Island was undertaken by the writer in 1952, the results of which are reported here. Emphasis has been placed on morphological variation in *Diphyllobothrium*.

MATERIALS AND METHODS

Field observations were centered around the Karluk Lake-Uyak Bay region of Kodiak Island (see map). Information on host-parasite relationships was obtained in the Karluk Lake region only. All experimental work was carried on at the laboratory in Anchorage, Alaska.

Adult cestodes were secured at the autopsy of bears, *Ursus arctos middendorffi* Merriam, which had been collected for the purpose or were otherwise available. Of particular importance is the material, consisting of 523 cestodes, taken from a young bear (about 4 years old, 430 lbs.) collected at Karluk Lake on September 10, 1952. This animal (autopsy No. 12062) was shot too late in the evening to permit immediate examination. The viscera were removed at once and exposed overnight at an air temperature of about 10° C. The following day, the living

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* Animal-borne Disease Branch, Arctic Health Research Center, Public Health Service, U. S. Department of Health, Education and Welfare, Anchorage, Alaska.

cestodes were taken from the intestine, washed in water, and killed individually by abrupt immersion in hot formalin-acetic acid-alcohol solution. Many additional cestodes were secured at the autopsies of other bears on Kodiak Island.

Cestodes to be mounted entire were stained with Semichon's acetic carmine or Ehrlich's acid hematoxylin stains, dehydrated in ethanol, and cleared in terpineol. Representative segments, after staining and dehydration, were cleared in terpineol and partially dissected before mounting. The removal of superficial layers of tissue, including the vitellaria, permitted an unobstructed view of the testes and details of the female genital organs. The dissection of cleared specimens under the low-power microscope seemed to give better results than the paraffin-embedding method of Markowski (1949, p. 108).

In connection with this work, comparative study was made of adult cestodes from naturally-infected Alaskan birds and mammals, which had been collected since 1949. This material was obtained at autopsy of the hosts, except that cestodes from man were eliminated through the use of drugs. Experimental infections of birds and mammals also yielded adult cestodes used in this study.

On Kodiak Island, plerocercoid larvae of *Diphyllobothrium* sp. were collected from spawning or spawned-out red salmon, *O. nerka* (Walbaum). Some of these larvae were fed to experimental animals, and the remainder fixed and preserved for study.

Six young black bears, *Ursus americanus* Pallas, were employed in infection experiments with plerocercoids from red salmon, since attempts to capture brown bear cubs for this purpose were unsuccessful. These bears had been caught in the spring, before weaning, and were maintained in a manner precluding accidental infection by fish-transmitted cestodes. The 6 bears were killed and autopsied after elapse of the desired periods of time following administration of larval cestodes.

Representative series of fishes of other species on Kodiak Island were examined for plerocercoids of *Diphyllobothrium* spp. The occurrence of plerocercoids is discussed further below, under "ecology." Comparative plerocercoid material was taken from steelhead (rainbow) trout, *Salmo gairdnerii* Richardson, from the Kenai Peninsula, southern Alaska.

RESULTS

Nearly all red salmon examined from the streams tributary to Karluk Lake harbored plerocercoid larvae of *Diphyllobothrium* sp., localized in cysts on the serosa of the stomach. Cestodes morphologically identical with those taken from bears on Kodiak Island developed when these larvae were fed to captive black bears. These results support the hypothesis that bears of Kodiak Island are infected with *Diphyllobothrium* sp. by ingesting plerocercoids in red salmon.

OBSERVATIONS ON MORPHOLOGICAL VARIATION

I. The adult cestode.

Since morphological characters, taken either singly or in combination, are apparently too variable to be relied upon, most of the species of the genus *Diphyllobothrium* are inadequately characterized and consequently difficult to identify with certainty. As pointed out by Stunkard (1949, p. 622), variation in the species of *Diphyllobothrium* is poorly understood, particularly in regard to the possible effect of differences in species of host. In the writer's opinion, the degree of normal mor-

phological variation in members of this genus may be comparable to that seen in certain cyclophyllidean cestodes (Rausch and Schiller, 1949; Rausch, 1952; Schiller, 1952).

The abundant material from bear No. 12062 had unusual value in demonstrating range of morphological variation under the uniform conditions of occurrence in a single host animal. This material has been used as the basis for evaluating characters

Map of northwest end of Kodiak Island, showing Karluk River drainage.

regularly considered in differentiating species of *Diphyllobothrium*. Each feature is discussed separately.

Scolex. The size and shape of the scolex are usually included in any description of a species of *Diphyllobothrium*. Wardle and McColl (1937, p. 164) pointed out that the scolex of *D. latum* may vary greatly in shape and size, as would be expected of such a motile organ. In the writer's material, shape of scolex depended upon the state of expansion or contraction at the time of fixation. Size of scolex

was relatively uniform, irrespective of strobilar size, and it is possible that average dimensions might in some cases have value in differentiating the present form from others. Among recent workers, both Markowski (1949) and Kuhlow (1953b) attached importance to size and shape of the scolex as having specific value.

Neck. The presence or absence of a neck, defined as the unsegmented portion of strobila following the scolex, is important in descriptions of *Diphyllobothrium* spp. In evaluating this character, Markowski (1949, p. 117) stated, "The 'neck' is of systematic value only in so far as it is present or absent, because the length of this part of the body varies considerably, even in the same species, and is in no way constant." Kuhlow (1953c, p. 21), in his detailed study of *D. dendriticum* (Nitzsch, 1824), found a neck to be consistently present. He also (1953b) made use of this character in differentiating other species studied.

The writer's material was unusual. A well-defined neck of variable length is present in young strobilae; however, the undifferentiated zone decreases in length as the cestode becomes older, and eventually disappears. In specimens from the black bears, 10-day-old strobilae had necks up to 5 mm. long. A 61-day-old specimen, measuring more than 3 meters in length, still had a short neck which was being eliminated through the process of segmentation. Old strobilae, without regard to size, were uniformly segmented to the base of the scolex. It is necessary to differentiate between incipient segmentation and transverse striation resulting from contraction in making these observations. This was readily accomplished in the writer's material because internal segmentation could be recognized, and the genital primordia were already visible in very young segments.

Elimination of the neck appears directly related to age of strobila, but not necessarily to its size. In bear No. 12062, strobilae of a great range in size occurred together. The smallest cestodes, some no longer than 500 mm., evidenced age; i.e., apolysis had occurred, normally-developed genital organs were present, and the eggs in the uteri were normal in size and number. Such small specimens showed no undifferentiated zone following the scolex. The same condition was observed in cestodes from other bears.

The persistence of the neck in the 61-day-old strobila was perhaps related to favorable growth conditions. Its host harbored only 2 other cestodes (*Taenia krabbei* Moniez, 1879) and a few ascarids (*Toxascaris* sp.), and had received abundant high-protein food.

Chizhova (1951) observed a similar situation in a cestode parasitizing gulls (*Larus argentatus* Pontoppidan), dogs, and man on Ol'khon Island, Lake Baikal. Although apparently of the same species, specimens with and without necks were found in both gulls and man. In both hosts 2 strobilar forms were observed, probably a manifestation of differences in age. Talyzin (1932, p. 724) stated, regarding the neck in *D. strictum* (Talyzin, 1932), that "Ein gut ausgepraegter Hals ist hier nicht vorhanden, jedoch beginnt die Segmentierung nicht sofort am Koepfchen (wie bei *D. minor*), sondern in einer Entfernung, welche der Laenge des Koepfchens gleicht." Chizhova identified 1 species on Ol'khon Island, though Talyzin recognized 3.

Kuhlow (1953c) did not observe a disappearance of the neck in *D. dentriticum.* Likewise, in the present study, specimens of *Diphyllobothrium* sp. obtained in dogs, foxes, bears, cats, and gulls, after feeding plerocercoids from steelhead (rainbow)

trout, showed in the neck little variation that could be related to strobilar age. These specimens ranged in age up to 100 days.

It appears that the presence of a neck depends upon age of strobila in some species of *Diphyllobothrium*. The undifferentiated zone is gradually eliminated as the cestode becomes older; the lack of a neck may indicate that the strobila is senile and no longer producing segments. It is difficult to evaluate the presence or absence of a neck until more is known of the normal life span and the possible effect of occurrence in different hosts. Possibly, cestodes of a given species of *Diphyllobothrium* are able to persist until they become senile in some hosts, but are eliminated relatively early from others. It is noteworthy that gulls often eliminate cestodes after a few weeks. Additional work on the comparative development of *Diphyllobothrium* spp. in different hosts is required to clarify these points. The presence or absence of a neck has no diagnostic significance in the cestode from Kodiak Island bears.

Size and form of strobila. Extreme differences in size of strobilae have been accepted as serving to distinguish some species of *Diphyllobothrium* from others. One gains the impression that a given species is relatively uniform in length of strobila, or at least that the range in length is not great. Such is often true when only a single species of host is involved, and when only a few cestodes are present.

In bear No. 12062, cestodes ranging in length from less than 500 mm. to more than 10 meters had attained a comparable degree of maturity. Crowding undoubtedly exerts a retarding influence on strobilar growth. This has been observed in certain species of cyclophyllidean cestodes, e.g., *Hymenolepis diminuta* (Rudolphi, 1819), and was demonstrated by Kuhlow (1953c, p. 20) in *D. dentriticum* from gulls. Wardle and Green (1941, p. 246) mentioned a similar observation on *D. latum* in dogs, and believed that superinfected animals soon lost most of their parasites. Petrushevskii and Tarasov (1933) found the strobilar length of *D. latum* in man to be inversely proportional to the number of cestodes comprising a given infection. In the above-mentioned bear, crowding appears to be the cause of the great range in size of strobilae.

It must be assumed that the largest cestodes from bear No. 12062 attained their size without, or despite, crowding. It is possible that they became established early in the summer, when red salmon were scarce, and developed from the first few larvae ingested, or possibly they were maintained over the winter from the previous summer. The former assumption is reasonable, considering that early growth is very rapid, and that strobilae as long as 4.5 meters had not undergone apolysis. Ward (1935) reviewed the literature on longevity of *D. latum* in man, and concluded that most records were unreliable and did not consider the possibility of recurrent infection. Leiper (1936), on the contrary, disagreed with Ward's conclusions and presented evidence gained experimentally that infections may persist for a period of years. Irrespective of the life span of *D. latum* in man, it is likely that bears lose their infections prior to, or during, the period of winter denning. This was first considered by Rush (1932), who concluded that seasonal diet changes preceding denning-time might cause expulsion of the cestodes. Cestodes of the genus *Diphyllobothrium* may be very sensitive to changes in the dietary habits of their host. Kuhlow (1953c, p. 20) observed that experimentally-infected gulls spontaneously lost their cestodes (*D. dendriticum*) during a period of improper

feeding. The possible seasonal loss of *Diphyllobothrium* infections by bears is discussed further below under "ecology."

Strobilae grow rapidly during early life. A 10-day-old strobila, obtained by feeding plerocercoids from red salmon to a black bear, averaged a daily growth increment of 96 mm., or 62 segments (total length 960 mm., 620 segments). A 61-day-old strobila of the same origin contained 932 segments, with a total length of 3.16 meters. Actual growth could not be determined because apolysis had already taken place, but the minimum growth had averaged 51 mm., or 15 segments per day. Wardle and Green (1941) found that strobilae of *D. latum,* in dogs, averaged a daily increment of 48 mm. for the 6- to 15-day period, and 61.5 mm. for the 15- to 30-day period. Apolysis occurred at about 24 days. For the same species, Petruschevskii and Tarasov (1933) reported an average daily increment of 47 mm. in dogs, and 150 mm. in man for the 6- to 36-day period. Kuhlow (1953b, p. 224) reported an average increase of 200 mm. per day for *D. latum.* Accurate growth data on another species, *D. dendriticum,* were also secured by Kuhlow (1953c). He found, in gulls (*Larus ridibundus* Linnaeus), that a strobilar length of 410 to 610 mm. was attained in 6 days, with a total of 350 to 450 segments. The average daily increase in strobilar length was about 70 to 100 mm., or 58 to 75 segments. The increase in length to the time of maturity averaged about 100 mm. per day. According to Kuhlow, length of strobila increased from the 6th to the 12th day by segment production, and apolysis was observed in the period of the 12th to 16th days.

In cestodes from Kodiak Island bears, growth of individual segments contributed more to attainment of large size than did production of segments. The latter occurred after maturity, but was so restricted as to do little more than balance losses taking place through apolysis. A length of 10 mm. or more was not uncommon for a segment in the larger strobilae. The longest specimen collected measured 11.7 meters, but contained only 1725 segments. Compared with the above-mentioned 61-day-old strobila, this was 3 times the length, but less than twice the number of segments. In some strobilae, width of segments may be great (as much as 23 mm.) without proportional increase in length. There is in all cases a progressive increase in the ratio of length/width as segments become older, but this varies in degree.

Two forms of segmentation, termed "primary" and "secondary," and related to age of the strobila, were described by Wardle and McColl (1937). Their observations were made on cestodes, identified as *D. latum,* from dogs. The primary type was characterized by elongate segments, while the secondary type possessed segments of greater width than length. Kuhlow (1953c) observed 2 strobilar forms in *D. dendriticum,* which he designated as "type A" and "type B," corresponding to the 2 types differentiated by Wardle and McColl. He found that mature examples of *D. dendriticum* could be reared from eggs taken from either strobilar type. Talyzin (1934) recognized 2 strobilar types in *D. minus* Kholodkovskii, 1916; these were designated as "type I" and "type II." Chizhova (1951) defined 2 strobilar types in a species of *Diphyllobothrium* from *Larus argentatus* in the Lake Baikal region; these were designated as "type A" and "type B." From these observations, one may presume that shape of segment is in part related to age of strobila.

Wardle and McLeod (1952, p. 606) stated, "In superinfested animals such

primary segmented worms may persist for several months, but usually within three weeks after the entrance of the plerocercoid larva into the mammalian host secondary segments begin to appear in the neck region, at first linear, three to four times as wide as long." Cestodes having the primary-type segmentation were not observed in "superinfested" bears. Experimentally-obtained 10-day-old strobilae contained some elongate terminal segments, but these were not representative of "primary strobilae" as originally defined.

The writer observed "primary-type" segmentation persisting as long as 100 days in dogs and foxes experimentally infected by feeding plerocercoids from *Salmo gairdneri*. In gulls (*Larus glaucescens* Naumann), segmentation progressed about as described by Kuhlow (1935c) for *D. dendriticum* in *Larus ridibundus*. In older strobilae, the segments comprising the posterior half were of greater length than width, but there was no evidence of "primary strobilae" as defined by Wardle and McColl, even in the youngest examples. All segments of cestodes from black bears were of greater width than length. This was true also for specimens expelled from man at about 65 days.

The "narrow tapeworm" of man, *D. strictum* Talyzin, 1932, was described on the basis of cestodes having elongate, primary-type segments. The original material was associated with cestodes identified as *D. minus*, already mentioned as existing in 2 strobilar forms, and *D. latum*. Markowski (1949, p. 122) expressed the opinion that *D. strictum* may be a synonym of *D. dendriticum*, on the basis of morphological similarities. Further work has been done in Buriat-Mongolia by Chizhova (1951), who stated that the cestode in gulls and dogs demonstrated 3 forms of strobilae. Two of these corresponded to the 2 types observed in *D. minus* by Talyzin (1934). The third form, designated as "group V," was observed in gulls and found to correspond morphologically with *D. strictum*. Chizhova (1951, p. 219) stated, ". . . the morphological structures of the parasitic worms of the sea gull and of mammals have so much in common that if it were not for such different environments, then they, without doubt, could be referred to one species." Chizhova reared procercoid larvae from eggs of both avian and human origin, and found them morphologically identical, with the same rate of development. Plerocercoids from naturally infected fishes were studied, and it was impossible to confirm the occurrence of a single species. When dogs and gulls were experimentally infected, however, the adult cestodes obtained were morphologically identical.

Present knowledge indicates that differences in host species may influence considerably the strobilar development of a given species of *Diphyllobothrium*. It does not appear to the writer that the primary-type of segmentation takes place during the growth of the cestode of bears on Kodiak Island. The youngest specimens, from naturally infected bears, had all segments of greater width than length, when total length of strobila ranged from 18 to 30 mm. Strobilae 50–60 mm. long were similar, and as length increased the segments were derived from the undifferentiated zone following the scolex. The presence of malformed terminal segments was noted, however, in 10-day-old strobilae ranging in length from 520 to 1050 mm. These are considered anomalous, since some contained the genital components of as many as 3 segments joined in such a way that future separation would undoubtedly be impossible. The significance of such segments is unknown. In reference to similar segments in *Diphyllobothrium* sp., observed in a mammal in Suri-

nam, Baylis (1947, p. 407) stated, "It seems possible that in the present case the host had accidentally acquired an infection with a species which was ill adapted to it, and that in consequence the parasites had developed abnormally." Markowski (1949, p. 112) observed multiple sets of genital organs in a single segment of *D. dendriticum;* he listed as a character common to avian species of *Diphyllobothrium* "very narrow elongate segments, becoming somewhat threadlike, . . . in the posterior region of the body" (p. 120). Kuhlow (1953c, p. 26) stated, "Die jugendliche Gliederkette ist durch das Auftreten schmaler, langer Proglottiden mit mehreren Genitalsaetzen und vielen Missbildungen characterisiert. Sie wird durch die Sekundaerstrobila ersetzt, deren Glieder schneller heranreifen und betraechtlich breiter als lang werden." Similar malformed segments have been described by other workers. These appear to be related in some way, at least in degree of malformation, to species of host in which they occur.

It is concluded that size of strobila *per se* has little diagnostic value. However, if data were available on age of strobila, size of infection, and species of host for the 2 or more species of *Diphyllobothrium* being compared, size of strobila would constitute a good diagnostic character. Such data can be obtained only through experimental infections.

Testes. Regarding the taxonomic value of the testes in species of *Diphyllobothrium,* Markowski (1949, p. 119) stated, "The number of testes is also very unreliable as a systematic criterion because in the same species, and even in a single worm, the maximum number may be twice as great as the minimum." Kuhlow (1953b) concluded that testes distribution in relation to the genital pore had taxonomic value in some species. In the writer's opinion, the situation is about as it is in *Andrya macrocephala* Douthitt, 1915 (see Rausch and Schiller, 1949) ; that is to say, neither number nor distribution of testes can be relied upon as being constant for a given species.

In the cestode from Kodiak Island, the testes ranged in number from about 400 to 1650 per segment. This represents a remarkable range, but it is related to segment size. The larger the segment, the more testes present. Size of testes would seem to have no taxonomic value.

Distribution of the testes is rather constant, there being a tendency for their restriction to 2 lateral, disconnected fields. However, the 2 fields are at times confluent anterior to the genital pore, near the anterior margin of the segment. The testes never extend into the area around the genital pore, and the testicular fields are never confluent posterior to the ovary. Dubinina (1951) found considerable variation in the distribution of the testes in segments from a single strobila of *Diphyllobothrium erinacei-europaei* (Rudolphi, 1819) from cats.

It is concluded that distribution of testes can be relied upon as being fairly uniform for a given species. Number of testes, however, is subject to a wide range of variation.

Ovary. Kuhlow (1953b) regarded shape of ovary to have unusual diagnostic value in the species studied by him. This could not be confirmed in material from Kodiak Island in which the ovary has a reticulate structure, with the 2 lobes connected by a narrow isthmus. Much variation was seen in the form and extent of the posterior and anterior horns of the ovarian lobes. Consequently, no specific shape is defined as being characteristic for this cestode. Aberrant structure was

also noted, usually in the form of secondary connections between the lobes, posterior to the normal one, and strand-like projections in many specimens extended from the antero-lateral margins of the organ.

Uterine coils. The number and arrangement of the uterine coils have been accepted as characters of importance in differentiating species of *Diphyllobothrium.* Both Markowski (1949) and Kuhlow (1953b) utilized these in defining the species studied by them. The taxonomic value of uterine development and the number of the lateral coils as specific characters has also been discussed in detail by Wardle and McColl (1937).

In the writer's material, the number of uterine coils is not diagnostic. The range in number of lateral coils is the same as in several other species. Variation in the position of the uterine coils results from differences in number of eggs present and in the state of contraction of the segment. It was often impossible to count the coils for this reason. The anterior extension of the uterus beyond the genital pore is important, but this character is not unique, for the Kodiak Island species.

Vitelline glands. In the Kodiak Island cestode, distribution of vitelline glands is basically the same as in other members of the genus. There is variation, however, in the arrangement of these glands around the female genital organs and the genital pore. Frequently they extend medially only as far as do the testes; in other examples, they overlap the uterine coils to a considerable degree. The vitelline glands are confluent anterior to the genital pore in some cases, and when this occurs, it is usually the same dorsally and ventrally. Sometimes dorsal confluence of the glands alone may be seen. The vitelline glands do not extend across the mid-line at the posterior margin of the segment, but they frequently extend farther mediad here than do the testes.

Distribution of the vitelline glands has taxonomic value, but enough material must be available to determine first the range in variation. Since a relationship exists between the distribution of the vitelline glands and the state of contraction of the segment, material to be compared should be handled and fixed uniformly.

Mehlis' gland. Some description of Mehlis' gland is generally included in diagnoses of species of *Diphyllobothrium,* and shape and size of this organ are listed as being diagnostic. Markowski (1949) did not attempt to utilize this character in differentiating avian species of *Diphyllobothrium.* Kuhlow (1953b), on the contrary, believed that the organ had a typical shape in each of the 4 species of *Diphyllobothrium* studied by him in detail.

In the Kodiak Island material, the Mehlis' gland appeared elliptical or crescent-shaped in dorso-ventral aspect, but often was so diffuse as to be hardly visible. Size of this organ has little value as a diagnostic character, and it is doubtful that shape is ever consistent in a given species.

Egg. Size of egg has been regarded as having unusual value in differentiating species of a given genus of cestodes. It has been found that size of eggs may be quite variable in species of cyclophyllidean cestodes (Rausch, 1952). This appears to be correlated with geographical distribution of specimens, rather than with differences in hosts parasitized (see Rausch, 1952; Schiller, 1952). A similar situation may exist with regard to variation in size of eggs of *Diphyllobothrium* spp.

The cestodes from Kodiak Island contained eggs which were fairly uniform in average size and in shape. The area involved was too small to permit reliable observations on variation related to geographical distribution. Eggs from cestodes

collected on the mainland were also uniform in size and shape, and closely resembled those from Kodiak Island. It is of interest that the reported sizes of eggs from various species of cestodes from the northern hemisphere fall within narrow limits.

According to present knowledge, differences of reasonable degrees in size and proportions of eggs may have value in distinguishing species of *Diphyllobothrium*. This can be evaluated only after confirmation of the specific status of the numerous species now regarded as valid (especially, those described by Russian workers).

Other characters. Regarding other characters used in differentiating species of *Diphyllobothrium,* Markowski (1949, p. 120) stated, "Some contemporary authors, *e.g.* Vergeer (1942) and Thomas (1946) introduce into their descriptions new features, among which are the measurements of the ootype, of the receptaculum seminis and of the genital apertures. These features are also of little systematic value. . . ." The writer has not found other characters of value in differentiating the adult cestodes of this genus.

II. The plerocercoid.

As a result of the work of Kuhlow (1953a), it appears possible to differentiate the plerocercoid larvae of certain species of *Diphyllobothrium*. The larvae of many species, however, have not been studied. Four points are worthy of consideration in attempting to characterize these larvae: 1) Localization in the body of the host; 2) Species of host; 3) External morphology; 4) Microscopic anatomy.

Kuhlow's work (1953a) has emphasized that the plerocercoid stages of certain species of cestodes have a characteristic locus in the host. Thus, the larva of *D. dendriticum* is free in the body cavity of the host; that of *D. osmeri* (von Linstow, 1878) occurs in cysts on the serosa of the stomach; the plerocercoid of *D. vogeli* Kuhlow, 1953, is found in peripheral liver tissue; and the larva of *D. latum* usually occurs unencysted in the musculature.

Infective plerocercoids of the Kodiak Island cestode encyst on the serosa of the stomach of the red salmon. In North America, encysted larvae have been reported by several investigators. Linton (1891b) observed encysted larvae in *Salmo clarkii lewisi* (Girard) in Wyoming; Cooper (1921) reported apparently encysted larvae in *Salvelinus marstoni* Garman, from Bernard Harbor, Northwest Territories; Fasten (1922) observed such larvae in *S. clarkii* Richardson, in Washington; Simms and Shaw (1931) reported encysted larvae in *Salvelinus fontinalis* (Mitchill) and *Oncorhynchus kisutch* in Oregon; Wardle (1932) collected encysted larvae from *O. nerka kennerlyi* (Suckley) in British Columbia; and Vergeer (1942) and Thomas (1946) conducted feeding experiments with encysted larvae from *Leucichthys* spp.

In Eurasia, Duguid and Sheppard (1944) and Hickey and Harris (1944) have recently established infections by feeding larvae from *Salmo trutta* Linnaeus. Unsworth (1944) carried on similar studies. Kuhlow (1953a) established infections by feeding to gulls encysted plerocercoids from the stomach of *Osmerus eperlanus* Linn. The investigations of Skvortsov and Talyzin (1940),[1] Petrushevskii and Boldyr (1935), Petrushevskii and Tarasov (1933), Tarasov (1936), and Chizhova (1951) have also been important in establishing relationships of encysted plerocercoids.

[1] Quoted in Chizhova (1951). The writer has not seen the original.

A high degree of host specificity appears to have been developed by some species. For example, Kuhlow (1953a) found the plerocercoids of *D. osmeri* only in *Osmerus eperlanus;* those of *D. vogeli* ocurred only in the liver of *Gasterosteus* spp., but plerocercoids of *D. dendriticum* occurred in the body cavity of the same species. Plerocercoids of *D. latum* have often been reported from fishes of the genera *Esox, Perca,* and *Lota.* They apparently occur less commonly in salmonids. The larvae of *D. minus* and *D. strictum* are encysted in fishes of the genera *Coregonus* and *Thymallus* (Pavlovskii, 1946; Chizhova, 1951).

Kuhlow (1953a) was able to differentiate plerocercoid stage larvae of 4 species of *Diphyllobothrium* (*D. dendriticum, D. osmeri, D. vogeli,* and *D. latum*) on the basis of their external characteristics. Several characters were useful in this; viz., shape and size of body, presence or absence of cuticular folds, state of scolex (evaginated or not), and, if present, the distribution of cuticular bristles. Plerocercoids of other species have been described, but often there has been no well-founded attempt to correlate this larval stage with the adult. Magath (1929) and Vergeer (1929) wrote extensively of the plerocercoid larva of *D. latum;* Linton (1891a, b) distinguished larvae of 2 forms which were thought to be plerocercoids of *D. cordiceps* (Leidy, 1871); Petrushevskii and Tarasov (1933) described plerocercoids from Karelian fishes; and Vergeer (1942) presented details of the plerocercoid of *D. laruei.* Numerous other descriptions in the literature are of no use at the present time, since it is unlikely that the forms could again be recognized.

The differentiation of *Diphyllobothrium* plerocercoids on the basis of microscopic anatomy has been dealt with by Kuhlow (1953a). Although he was concerned with only 4 species, his method appears to be reliable and should be an important aid in elucidating some of the existing taxonomic problems. He concluded that characteristics of the frontal glands, arrangement of muscle layers, length and disposition of cuticular bristles ("Kutikulaborsten"), and relative thickness of body layers serve to differentiate species of plerocercoids. Kuhlow believed that the cuticular bristles had been overlooked by earlier workers. Linton (1891a, p. 76), in a paper probably not available to Kuhlow, stated in describing the smaller plerocercoid from trout in Wyoming, "The musculature and vascular systems are substantially the same as in the larger specimens. There is, however, an epidermal layer present which was absent from the larger specimens. This epidermis appears in transverse sections as a border of short, curved, hair-like processes springing from a thin basement layer, which separate easily from the cuticle." Subsequent workers failed to take advantage of Linton's observations.

CONCLUSIONS

The Kodiak Island cestode perhaps can be characterized according to structural and ecological peculiarities of both the adult and plerocercoid stages. Our present knowledge renders it impossible to confirm that this form is specifically identical with any previously-described species. Consequently, in order to characterize it and make available a name by which it may be designated, it is provisionally described as a new species. Since the intestine of the brown bear appears to constitute the most favorable habitat, and since maximum development is attained in this host, the name *Diphyllobothrium ursi* n. sp. provisional is proposed.

Diphyllobothrium ursi n. sp. provis.
(Figs. 1–5 and 7–8)

Diagnosis (adult stage): Strobilar length up to 11 meters; maximum width 23 mm.; maximum number of segments about 1800. Neck absent in mature strobilae. All segments of greater width than length. Anterior segments containing eggs with length/width ratio of about 1:8; older segments approaching square shape, with length/width ratio about 1:1.5. Strobilar margins only slightly serrate. Scolex relatively small and muscular, measuring 1.0 to 1.5 mm. long, by about 0.750 mm. thick; width (lateral view) up to 1.5 mm.; shape variable. Bothria extending full length of scolex. Adjacent part of strobila wider than scolex. Genital pore situated in anterior half of segment, often near anterior margin; surrounded by elevated area covered by papilla-like protrusions. Ovary, situated at posterior margin of segment, consisting of 2 distinct lobes connected by narrow isthmus near middle. Ovarian structure reticulate; anterior and posterior horns of lobes variable in position and degree of development. Vagina running from ovary directly anteriad to genital pore, superficial and ventral until just posterior to genital pore, there turning abruptly dorsad and extending nearly to dorsal wall of segment, then turning abruptly ventrad, paralleling cirrus sac to genital sinus and emptying into floor of sinus just posterior to male opening. Primordia of uterus visible in earliest segments (60 to 80 segments, or as little as 5 mm., from scolex). In mature segments, posterior portion of uterus much coiled; anterior portion, containing fully-developed eggs, consisting of 4 to 8 loops on each side of mid-line; anterior-most loops often extending well beyond genital pore. Pattern of uterus depending upon degree of segmental contraction. Uterine pore situated just posterior to genital pore, on mid-line. Fully-developed eggs elongate, measuring 56 to 69 microns long by 39 to 49 microns wide (av. about 65 by 43 microns). Vitellaria numerous, variable in size; distributed in lateral, disconnected fields, or at times confluent at anterior margin of segment; in latter case, they may be confluent dorsally and separate ventrally, or confluent both dorsally and ventrally. Vitellaria sometimes overlapping uterine coils, depending somewhat upon state of contraction of segment. Vitellaria superficial, with long axes directed dorso-ventrally. Major vitelline ducts variable in number and size, joining to form single, primary duct of relatively large diameter, latter joining oviduct near and dorsal to isthmus of ovary. Mehlis' gland diffuse, of variable size, and usually appearing oval or crescent-shaped when viewed dorso-ventrally. Testes abundant, up to 1650 per segment, of variable diameter, but usually about 150 microns. Testes usually disposed in disconnected lateral fields, or extending across mid-line anterior to genital pore, never extending across mid-line posterior to ovary. Cirrus sac piriform, large, up to 1.3 mm. long by 575 microns wide; distal end directed anteriad; ejaculatory duct coiled. Propulsion vesicle well developed; spherical in shape, measuring up to 650 microns in diameter, and situated just posterior to distal end of cirrus sac. Male genital opening situated in anterior part of floor of genital sinus.

Host: Ursus arctos middendorffi Merriam.

Locality: Kodiak Island, Alaska.

Habitat: Small intestine.

Type: Slides, No. 49355, containing portions of adult strobilae, have been deposited in the Helminthological Collection of the U. S. National Museum.

Diphyllobothrium ursi n. sp. provis. Plerocercoid larva.
(Figs. 6 and 10, 11, 12)

Diagnosis (plerocercoid stage): Excysted larvae 5 to 20 mm. long. Scolex evaginated; about 1.0 mm. wide, and oval to cordate in lateral view, and about 0.5 mm. thick; about same width as adjoining body. Body tapered toward either end, somewhat flattened dorso-ventrally; maximum width anterior to middle about 1.0 mm.; posterior end terminating bluntly. Dorsal and ventral median grooves present. Cuticle showing few folds, or none. Integument of plerocercoid, including scolex, covered with cuticular bristles of uniform length, 8 to 9 microns long. Cuticle about 4–5 microns thick; subcuticular zone about 8 microns thick. Next deeper zone, containing calcareous corpuscles, 24 to 30 microns thick. Calcareous corpuscles spherical, 3 to 4 microns in diameter. Subcuticular muscle fibers arranged singly; muscles of parenchyma arranged in large bundles, about 30–50 microns thick. Frontal glands well developed, arranged in dorsal and ventral masses, connected by thin isthmus; not extending into neck.

Host: Oncorhynchus nerka (Walbaum).

Habitat: In cysts on serosa of stomach.

Slides of entire and sectioned specimens have been deposited in the Helminthological Collection of the U. S. National Museum, No. 49356.

DISCUSSION

Species of *Diphyllobothrium* reported from marine mammals are not considered here. The writer has at hand a fairly representative collection from *Odobenus, Erignathus, Phoca,* and *Eumetopias,* and none of these closely resembles *D. ursi* n. sp. Neither does any described species, including those assigned by some authors to other genera (see Wardle and McLeod, 1952) resemble the present species. Diadromous and anadromous fishes bearing plerocercoids of *D. ursi, D. osmeri,* and possibly of other species, are undoubtedly available to piscivorous marine mammals. Mueller's statement (1937), ". . . it seems rather improbable that the same genus of cestode will be found in both the porpoise and land-dwelling mammals," is not necessarily valid in view of the lack of host specificity demonstrated by some species of *Diphyllobothrium.*

The species described here resembles closely some previously described species of *Diphyllobothrium.* It cannot be differentiated with certainty from some of these, but neither can it be established that they are identical. The following species are compared:

D. latum (Linnaeus, 1758). Parasitic in man, dog, and possibly bear; of circumboreal distribution. *D. ursi* n. sp. can be separated on the basis of larval characters, including morphology, host parasitized, and habitat in the host. The broad range of morphological variation observed in the adult *D. ursi* n. sp. makes its differentiation difficult from the adult of *D. latum.*

D. minus Kholodkovskii, 1916, and *D. strictum* Talyzin, 1932. Parasites of man, obtained from Buriats on Ol'khon Island in Lake Baikal. Although these 2 species were based on differences in strobilar form, they probably constitute a single species. According to a figure in Pavlovskii (1946, p. 290), the plerocercoid of *D. minus* has an everted scolex and a blunt, possibly invaginated posterior end. The body diameter is relatively great in relation to length, and cuticular folds are present. The work of Chizhova (1951) indicates that a single form, capable of infecting man, dogs, and gulls, exists in the region of Ol'khon Island. Additional work is needed to determine its status. Markowski (1949) regarded *D. strictum* as a synonym of *D. dendriticum,* while Kuhlow (1953b) was of the opinion that both *D. minus* and *D. strictum* are dwarfed forms of *D. latum.* The present species is readily differentiated from these forms on the basis of both larval and adult characters.

D. tungussicum Podiapolskaia and Gnedina, 1920. A parasite of man, obtained from Iakuts and Tungus from the lower Tungus and Lena region. This species closely resembles *D. latum,* of which it was thought by Kuhlow (1953b) to be a dwarfed form. A mature segment was figured by Pavlovskii (1946, p. 294). The status of this cestode is indefinite, and *D. ursi* n. sp. as yet cannot be differentiated adequately from it.

D. skrjabini Plotnikoff, 1932. Parasitic in man in the German National Okrug, lower Pechora River, and reported from dogs at Sverdlovsk. According to Pavlovskii (1946, p. 293), this cestode, with a maximum length of only 2.6 meters, has as many as 2700 segments. *D. ursi* n. sp. is differentiated from it on this basis. The life cycle of *D. skrjabini* is not known.

D. nenzi Petrov, 1938. A parasite of man from the lower Pechora River region. The only description available to the writer is that of Pavlovskii (1946, p. 294;

fig. p. 296). According to this description, no segmentation is visible for as much as 320 mm. posterior to the scolex. The testes extend across the mid-line posterior to the ovary. *D. ursi* n. sp. is distinguished by differences in form of strobila and distribution of testes.

D. giljacicum Rutkevich, 1937. Parasitic in man, from Giliaks of the west coast of Sakhalin. This cestode occurred with *D. latum,* and may be identical with it. With a maximum strobilar length of only 2.6 meters, it possesses as many as 1830 segments. Eggs first appear in the uterus about 980 mm. from the scolex. According to this information, *D. ursi* n. sp. differs in form of strobila, and in rate of maturation. Additional information on *D. giljacicum* is needed before determination of its status.

D. luxi Rutkevich, 1937. A parasite of man, from Iakuts of the eastern shore of Sakhalin. This species resembles *D. nenzi* and *D. giljacicum* in the apparent slow rate of maturation of the genital organs and the relatively great number of segments (1662) for the length of its strobila (1.5 meters). This species may not be valid, but adequate data are lacking. *D. ursi* n. sp. can be differentiated from it, apparently, on the basis of the form of its strobila and rate of maturation.

D. cordiceps (Leidy, 1871). A parasite of gulls, white pelican, and bears, in Wyoming. The only description that is fairly complete is that of Ward (1918, p. 432), which has no value for taxonomic purposes. Through the kindness of Dr. E. W. Price, Animal Disease and Parasite Research Branch, Agricultural Research Service, U. S. Department of Agriculture, the writer had available for study 2 adult strobilae and some plerocercoids designated as *D. cordiceps.* These were from black bear and *Salmo* sp. ("rainbow trout"), respectively, the former from Yellowstone Park and the latter from Oregon.

Skinker (1932, p. 162) concluded that ". . . on the basis of morphology of the material available for study, *D. cordiceps* for the present must be considered a synonym of *D. latum.*" At the same time, Scott (1932, p. 162) concluded that the cestode in Yellowstone Park bears was *D. cordatum* (Leuckart, 1863), but found that eggs were indistinguishable from those of *D. latum,* and that the larvae appeared to be identical. Woodbury (1935) was unable to infect himself by ingesting both free and encysted larvae from *Salmo lewisi* [sic], and concluded that the species represented probably was not *D. latum.* More recently, Markowski (1949) concluded that *D. cordiceps* is a synonym of *D. dendriticum.* Kuhlow (1953b, p. 230) was not willing to agree with Markowski's conclusion, however, and emphasized the need for further study.

Unfortunately, Linton's description of the larvae (1891a, b) does not elucidate the problem, since he apparently was dealing with 2 species. His descriptions were adequate to indicate that he had a long form (up to 15 cm.) which showed transverse striae, and a small, encysted form. The latter was described by Linton (1891b, p. 339) thus: "The smaller larvae up to a centimeter or more in length are lanceolate, flattish, tapering rather abruptly and uniformly to each end." His description of the microscopic anatomy of this form was insufficient for present purposes. In any event, it is certain that a long plerocercoid similar to that of *D. latum* (as described by North American workers) were present, along with a small one similar to that of *D. ursi* n. sp. Feeding experiments involving both forms will be necessary to determine which is the larva of *D. cordiceps.*

From the adult cestode, *D. ursi* n. sp. can be differentiated by its larger, relatively long eggs (av. size in *D. cordiceps*: 56 by 43 microns), and by a more restricted distribution of the vitellaria (see Fig. 9). Other apparent differences cannot be evaluated at the present time.

D. laruei Vergeer, 1942. Described from adult and immature strobilae obtained by feeding dogs and cats encysted plerocercoids from *Leucichthys* spp. in the Great Lakes region. Attempts to infect young gulls were unsuccessful (Vergeer, 1942, p. 373).

Markowski (1949, p. 123) concluded that *D. laruei* is a synonym of *D. dendriticum*. Kuhlow (1953b) regarded *D. laruei* as distinct from *D. dendriticum*, and concluded that *D. oblongatum* Thomas, 1946, probably represents the primary strobila of *D. laruei*. *D. oblongatum* was regarded by Markowski as a synonym of *D. dendriticum*.

The description of the plerocercoid of *D. laruei* by Vergeer (1942) is too incomplete for present needs. Externally, however, it resembles larvae of *D. osmeri*, *D. vogeli*, and of *D. ursi* n. sp. Additional work is needed to determine the status of *D. laruei*.

D. ursi n. sp. differs from the published description of *D. laruei* in having a much larger egg (av. size in *D. laruei*: 47 by 33 microns), and a restricted distribution of the testes, which in *D. laruei* extend across the mid-line posterior to the ovary. Other apparent differences cannot be evaluated at the present time; among these, shape of ovary may be significant.

D. ditremum (Creplin, 1825). Recently studied in detail by Markowski (1949) and Kuhlow (1953b), *D. ditremum* apparently parasitizes only piscivorous birds, particularly colymbiform and pelecaniform species. *D. ursi* n. sp. differs from *D. ditremum* in having larger eggs, many more testes, and much larger strobila. Host species may also be diagnostic.

D. dendriticum (Nitzsch, 1824). Recently studied by Markowski (1949) and Kuhlow (1953a, c), this species is commonly found in gulls, but dogs and other mammals have been experimentally infected. Kuhlow has provided a good knowledge of normal morphological variation in this species.

D. ursi n. sp. is distinguished from *D. dendriticum*, in the adult stage, by its larger eggs, possessing more testes, and larger strobila. However, there is no information on the development of *D. dendriticum* in bears. In the plerocercoid stage, *D. ursi* n. sp. differs in having a relatively smooth cuticle, cuticular bristles over the entire scolex, and in thickness of subcuticular layers.

D. osmeri (von Linstow, 1878). Natural final host not known; thought by Kuhlow (1935b p. 208) to be a marine mammal, since the plerocercoid occurrs in an anadromous fish. Experimentally obtained in gulls.

In the adult stage, *D. ursi* n. sp. differs from *D. osmeri* in having the gravid uterus extending beyond the genital pore, more testes, larger eggs, and a much larger strobila. In the plerocercoid stage, *D. ursi* n. sp. has much shorter cuticular bristles and differently arranged subcuticular muscles.

D. vogeli Kuhlow, 1953. A parasite of gulls in Germany. The adult *D. ursi* n. sp. has more testes, a differently shaped ovary, larger eggs, and a much larger strobila. The plerocercoid stage of *D. ursi* n. sp. has much shorter cuticular bristles, and different relative proportions of the subcuticular layers.

Diphyllobothrium sp. indet. Adult cestodes were experimentally obtained in gulls and certain species of mammals, including man, by feeding plerocercoids from steelhead (rainbow) trout, *Salmo gairdnerii,* collected on the Kenai Peninsula, southern Alaska. Excepting young trout, up to 150 mm. in length, all were heavily infected with encysted plerocercoids. These larvae closely resembled the plerocercoid of *D. ursi* n. sp. in most details. However, development of the adult stage is greatly influenced by the host. Differences in size, form of strobila, rate of development, and other features do not permit the conclusion that this form and *D. ursi* n. sp. are conspecific. Mature segments from a variety of host species are shown in figs. 13–17. Any decision regarding the 2 forms must await further study of material from experimentally infected animals.

Possibly, the situation in Alaska (and other parts of North America) may be the same as that observed by Chizhova (1951) on Ol'khon Island, where apparently a single species of *Diphyllobothrium* occurs in both birds and mammals, but the considerable morphological variation results in part from differences in hosts.

Whether *D. ursi* n. sp. is capable of infecting man has not yet been determined. It is hoped that this phase of the problem can be investigated on Kodiak Island in the future. It is well known that fish tapeworm infection is epidemic in the lower Kuskokwim River region of Alaska (Hitchcock, 1950), but the species of *Diphyllobothrium* involved cannot be identified as yet. A study of the characters of plerocercoid larvae, with experimental infections of various animals, should clarify this problem. Mature segments of cestodes from man in Alaska are shown in figs. 18–20. On the basis of the strobilar stage, this species cannot be differentiated from *D. ursi* n. sp.

NOTES ON THE ECOLOGY OF *D. ursi* N. SP.

Around Karuk Lake, red salmon make up an important part of the diet of bears during July and August, and to a lesser degree in September. As the salmon become available, the bears congregate along the smaller streams to feed on them. Most of the red salmon eaten by these mammals are spawned-out or already dead, since the more recently arrived individuals are very active and escape by swimming downstream when disturbed. During the several weeks the bears are feeding on red salmon, large numbers of plerocercoids are ingested. These establish themselves in the small intestine of the bears, beginning about 2 meters below the stomach, and rapidly attain sexual maturity.

Depleted portions of strobilae appear in the feces of the bears as early as August 1, but these are seen in increasing quantities as the season progresses. When berries become available [particularly *Sambucus racemosa* L. and *Viburnum edule* (Michx.)] around September 1, the bears leave the streams to feed on vegetation. However, salmon are eaten by them until December; these are either red salmon spawning along the shores of Karluk Lake, or carrion. Late in the fall, large quantities of cestodes are seen in the feces, mixed with the remains of vegetation.

It has not been determined whether *Diphyllobothrium* spp. are capable of overwintering in the intestine of the bear. Rush (1932, p. 275) stated, "The absence of the Russian broad tapeworm in the bears trapped in October at a distance of more than 20 miles from the Lake may indicate one of two conditions, viz: the infestation in the Park may not extend beyond the Lake district, or, the bear may

have a natural means of ridding themselves of parasites before going into hibernation. There is some evidence that most parasites are very specific in their diet and it seems reasonable that the broad tapeworm could not subsist in the intestines of a bear after it goes on a grass diet, which it does in the fall months." There is some evidence to support the hypothesis that bears and true hibernators lose their helminth parasites in the fall. At the writer's request, Mr. Alf Madsen, an experienced professional guide, examined bears shot by hunters on Kodiak Island during the fall of 1953. He stated that 11 bears killed during September in the vicinity of Karluk Lake and Uganik Bay all harbored cestodes, but 6 animals killed in the same locality after October 20 were negative. On the contrary, an old female bear killed by Mr. Russell R. Hoffman, Biologist, U. S. Fish and Wildlife Service, on December 26 at Karluk Lake, harbored about 500 cestodes. On Kodiak Island, the bears often do not den until December, and sometimes not at all. They usually emerge during April.

Bears killed soon after emergence from the winter den have a rectal obstruction, composed of dehydrated remnants of plant material, which probably has been retained throughout the time of denning. According to Mr. Madsen and others, bears killed in the spring, before elimination of this obstruction, never harbor cestodes.

In June, 1953, the writer attempted to gain further information on survival of cestodes in denning bears. It was assumed that new infections could not take place until the beginning of the salmon runs in July. Five bears, killed along Uyak Bay, were examined, viz. a yearling, 2 two-year-olds, and 2 adults. All were negative for cestodes. Two animals contained a few immature ascarids which had undoubtedly been acquired since emergence from the winter den.

Since red salmon do not spawn in the streams emptying into Uyak Bay, and there is no definite information on the distance individual bears may travel, it is uncertain that these bears were ever exposed to infection through the eating of red salmon. These findings, therefore, were inconclusive. There was no opportunity to obtain bears from around Karluk Lake at the time. The finding of cestodes in bear feces along Sulua Creek, near Olga Bay, several miles from any drainage used by red salmon, seems to indicate that considerable movement of the animals may take place. The greatest activity is seen during the breeding time (May–June).

The writer has been told by Nunamiut Eskimo of northern Alaska that bears lose their parasites in the fall before denning. These people, who are accurate observers, were impressed by the abundance of ascarids in the bears killed in late summer. It was concluded by Dubinin and Leshkovich (1945) that marmots, *Marmota sibirica* Radde, true hibernators, eliminate in the fall the ascarids which they harbor during the warm months. They stated (page 377), "In the beginning of October, the worms left and the marmots went into hibernation." Bears probably lose their cestodes in the fall when they stop feeding. The change in diet from salmon to vegetation may be enough, in itself, to bring this about.

During the time the bears are feeding on salmon, they defecate freely in and along the streams, so that large numbers of cestode eggs reach an environment favorable for development. The eggs themselves, or the coracidia, are carried downstream into Karluk Lake, which has an abundant copepod fauna. The writer has made no observations on this part of the life cycle of *D. ursi* n. sp., but there

is much information on the zooplankton of Karluk Lake as a result of the studies of Juday, Rich, Kemmerer, and Mann (1932).

The red salmon, after hatching, usually spend 3 years in the lake, during which time they feed largely on zooplankton. They would undoubtedly be infected during this time through the ingestion of copepods containing procercoid larvae of *D. ursi* n. sp. The role of copepods in the diet of red salmon fry has been discussed by Foerster (1934). The 3-year-old red salmon leave the fresh water and enter the sea. Here they spend either 2 or 3 years, and then return to spawn in the drainage where they hatched. By the time of their return, they harbor infective plerocercoids which are ingested by the bears.

Four other species of salmon occur in the Karluk River drainage. The king salmon, *O. tschawytscha* (Walbaum) spawns in the Karluk River, as does the dog salmon, *O. keta* (Walbaum). The silver salmon, *O. kisutch,* spawns mainly in the Karluk River and in the lake itself. The pink salmon, *O. gorbuscha* (Walbaum), spawns in the Karluk River and also in some of the tributaries used by the red salmon. Of these, the fry of pink and dog salmon enter salt water immediately upon hatching. The fry of king salmon feed upon copepods, but it is not known to the writer whether this species harbors plerocercoids of *D. ursi* n. sp. The fry of silver salmon do not feed on plankton. It is of interest that Euguchi (1934) obtained plerocercoids regarded as *D. latum* from both *O. gorbuscha* and *O. keta* in Japan. He stated (p. 4149), "The occurrence of plerocercoids is very common in *O. masou* and *O. gorbuscha,* but a rare phenomenon in *O. keta.*" The former species does not occur in North America, but it appears improbable that the fry of *O. gorbuscha* remain long enough in fresh water to become infected from eating fresh-water copepods. An attempt to infect experimentally birds and mammals using larvae from *O. gorbuscha* might be enlightening. Examination of a series of pink salmon disclosed no larvae of *Diphyllobothrium;* however, they were taken from a stream not used by red salmon and not frequented by bears. Further observations on this species and on king salmon are needed.

The examination of series of other fishes from Karluk Lake [viz., *Salvelinus malma* (Walbaum), *O. kisutch, Salmo gairdnerii, Cottus* sp., and *Gasterosteus aculeatus* Linnaeus] gave uniformly negative results. A large series of *S. gairdnerii* was available, and the fact that no plerocercoids were found in them would support the view that *D. ursi* n. sp. is specifically distinct from the cestode occurring on the mainland. To the writer's knowledge, no observations have been reported on the occurrence of *Diphyllobothrium* spp. in bears (*Ursus arctos*) in the regions of northeastern Asia where they also feed on spawning salmon.

CONCLUSIONS

From observations made on cestodes from Kodiak Island bears, it has been found that a wide range of variation is to be expected in morphological characters heretofore used in differentiating species of the genus *Diphyllobothrium* Cobbold, 1858. It is concluded that species cannot be effectively differentiated through use of morphological features of the adult cestodes alone. The following points seem pertinent if adequate information is to be obtained on a given species:

The plerocercoid should be studied in detail, including host-species, habitat, and morphology. An attempt should be made to rear adult cestodes in a variety of

piscivorous birds and mammals, so that comparative material of known age can be obtained. Animals of a given species should be fed uniformly, to avoid any possible effect of changes in diet. Before comparing morphological features, observations should be made on rate of growth of strobila, time required for attainment of sexual maturity, onset of apolysis, and, if possible, normal life span. Kuhlow's (1953c) investigation of *D. dendriticum* could serve as a model for a study of this type.

Data of the kinds stated should eventually permit an evaluation of the named species of *Diphyllobothrium*, many of which can hardly be valid. Cestodes occurring in man fall into 2 main groups: those whose plerocercoid larvae occur encysted in salmonid fishes, and those whose larvae occur non-encysted in fishes of other families. Most of the species of *Diphyllobothrium* in boreal Eurasia and North America, for which the plerocercoid stages are known, fall into the first group. Fishes of the same species occur on both sides of Bering Strait, and it is probable that some of the same species of *Diphyllobothrium* will be recognized in the northern regions of both continents.

It would seem at first that species of *Diphyllobothrium* which occur in marine mammals would be difficult to study, and this is undoubtedly true for some. However, several species of anadromous and diadromous fishes are available to marine mammals. In addition, some fishes move freely between fresh water and salt water. An example of this is a whitefish, *Leucichthys sardinella* (Valenciennes), from which, incidentally, the writer has collected at Point Barrow the plerocercoid stage of a species of *Diphyllobothrium* harbored commonly by seals. Some marine mammals, moreover, enter fresh-water streams. For example, the harbor seal, *Phoca vitulina* Linnaeus, is often seen around river mouths, and the white whale, *Delphinapterus leucas* Pallas, sometimes travels several miles up streams. Further study of these interrelationships should be particularly productive.

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Explanation of Plates

Plate I

Fig. 1. *Diphyllobothrium ursi* n. sp., mature segment from naturally infected brown bear, Kodiak Island, Alaska.

Fig. 2. *D. ursi* n. sp., mature segment from naturally infected brown bear.

Fig. 3. *D. ursi* n. sp., scolex of mature cestode from naturally infected brown bear.

Fig. 4. *D. ursi* n. sp., mature segment from naturally infected brown bear.

Fig. 5. *D. ursi* n. sp., sagittal section through mature segment, showing relationships of genital ducts. From naturally infected brown bear.

Fig. 6. *D. ursi* n. sp., plerocercoid larva from red salmon, excysted.

Fig. 7. *D. ursi* n. sp., 10-day-old mature segment from experimentally infected black bear.

Fig. 8. *D. ursi* n. sp., 61-day-old mature segment from experimentally infected black bear.

Plate II

Fig. 9. *D. cordiceps* (Leidy, 1871), mature segment from naturally infected black bear, Yellowstone Park, Wyoming.

Fig. 10. *D. ursi* n. sp., scolex of plerocercoid, lateral view.

Fig. 11. *D. ursi* n. sp., segment of cross-section of plerocercoid; drawn from 5 micron section, stained with hematoxylin-eosin.

Fig. 12. *D. ursi* n. sp., scolex of plerocercoid, dorso-ventral view.

Fig. 13. *Diphyllobothrium* sp., 107-day-old mature segment from experimentally infected arctic fox, *Alopex lagopus* L.

Fig. 14. *Diphyllobothrium* sp., 65-day-old mature segment from experimentally infected man.

Fig. 15. *Diphyllobothrium* sp., 56-day-old mature segment from experimentally infected dog.

Fig. 16. *Diphyllobothrium* sp., 10-day-old mature segment from experimentally infected gull, *Larus glaucescens* Naumann.

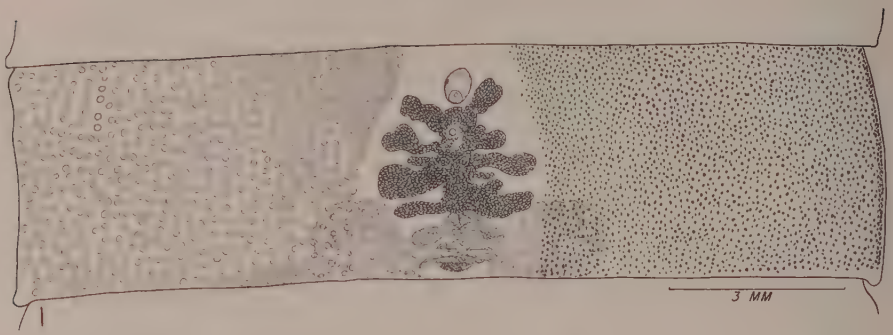
Fig. 17. *Diphyllobothrium* sp., 107-day-old mature segment from experimentally infected black bear.

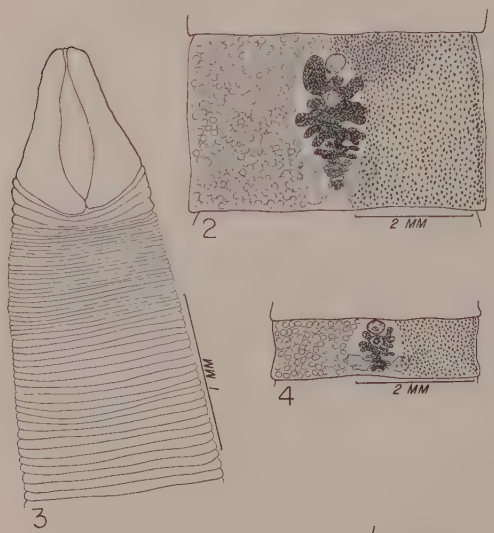
Fig. 18. *Diphyllobothrium* sp., mature segment from naturally infected man, Kotzebue, Alaska.

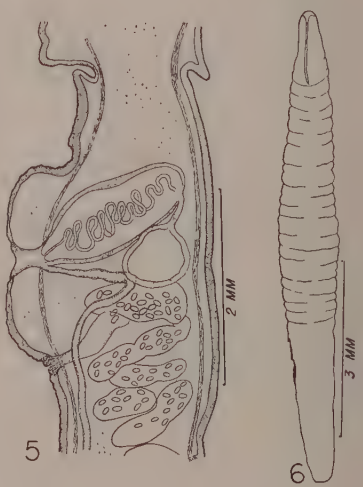
Fig. 19. *Diphyllobothrium* sp., mature segment from naturally infected man, Nelson Island, Alaska.

Fig. 20. *Diphyllobothrium* sp., mature segment from naturally infected man, Bethel, Alaska.

PLATE I







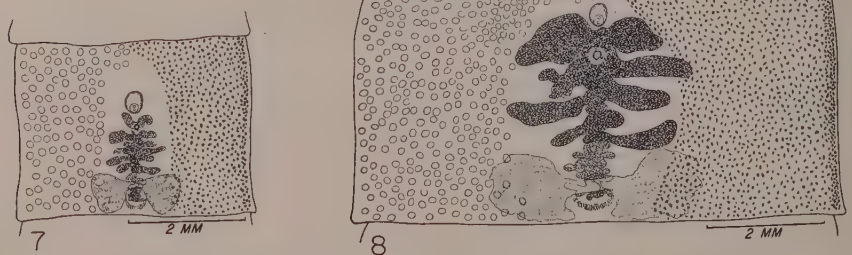
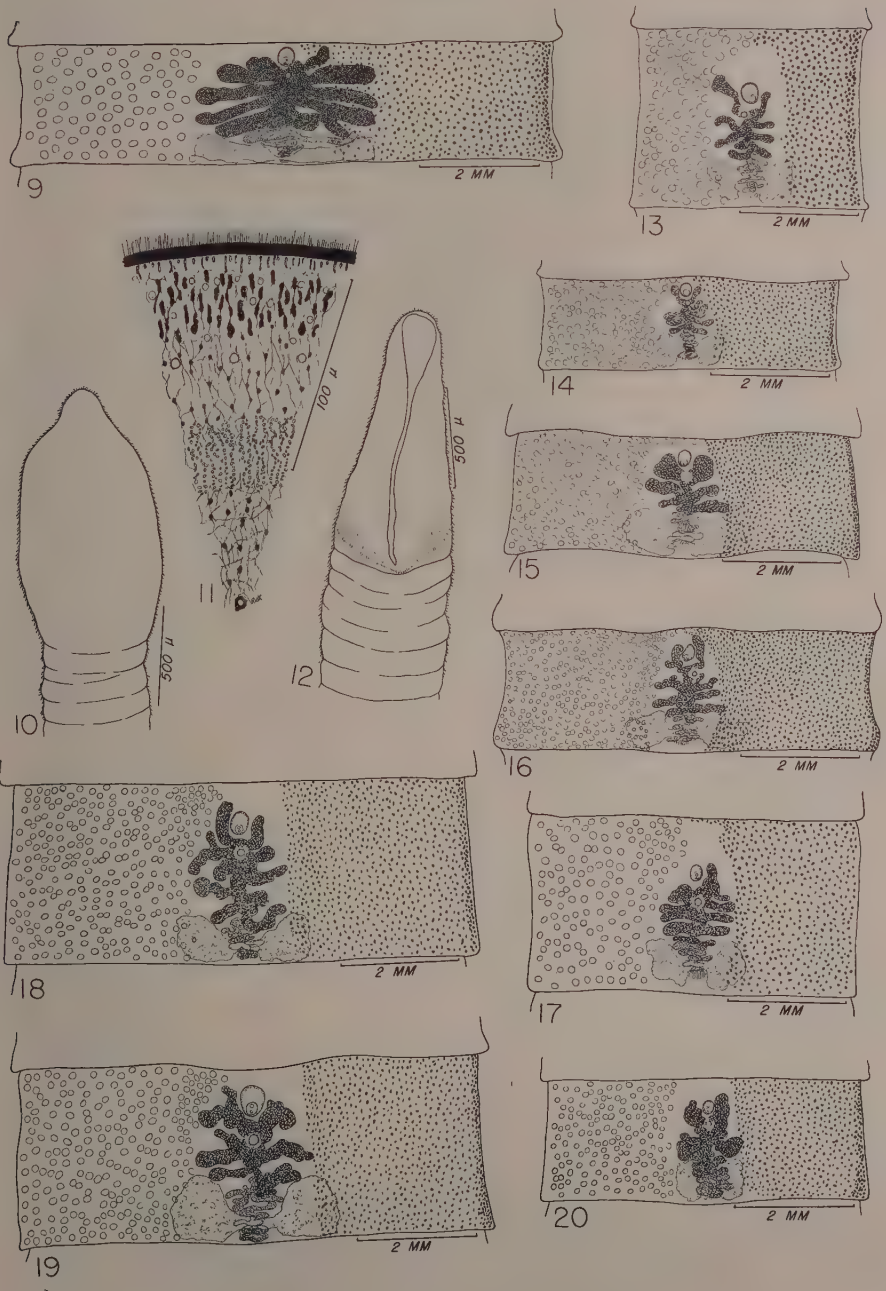


PLATE II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A DESCRIPTION OF *PARAFIMBRIARIA WEBSTERI* N. G., N. SP., A CESTODE FROM GREBES, AND NOTES ON THREE SPECIES OF *HYMENOLEPIS*

MARIETTA VOGE

Department of Infectious Diseases, School of Medicine
University of California, Los Angeles

AND

CLARK P. READ*

Department of Zoology, University of California, Los Angeles

Study of cestodes collected from the grebe, *Colymbus nigricollis*, taken at Guardian Angel Island, Gulf of California, and Salton Sea, Imperial County, California, during 1953, revealed that 3 species of hymenolepidid cestodes were represented. One of these, a member of the subfamily FIMBRIARIINAE, is considered to represent a new genus. The remaining material from the grebes is referred to 2 previously known species, *Hymenolepis furcigera* (Krabbe, 1869) and *H. podicipina* Szymánski, 1905. Notes are presented on this latter material and on a third species, *Hymenolepis recurvirostrae* (Krabbe, 1869), collected from *Recurvirostra americana*, taken at Newport Bay, California.

Worms were studied as whole mounts fixed in Bouin's fluid and stained with dilute Ehrlich's haematoxylin.

Appreciation is expressed to Mr. Joseph Sefton, who facilitated the collection of Gulf of California material by one of us (C.P.R.), and to Drs. Wade Fox and Thomas Howell, who made material available to us.

Parafimbriaria websteri n.g., n. sp.

Specific diagnosis: FIMBRIARIINAE: Pseudoscolex lacking. Scolex with 4 suckers and rostellum bearing 10 hooks. Scolex diameter 120 to 138 microns; sucker diameter 41 to 52 microns; everted rostellum diameter 51 microns; hook length 36 to 40 microns. Differentiation of sexual apparatus beginning 300 to 400 microns from scolex. Inner longitudinal musculature consisting of approximately 100 bundles. Outer longitudinal musculature rudimentary or absent. Excretory system consisting of 4 longitudinal ducts, with irregularly distributed and branching connecting ducts. Genital pores unilateral. Three spherical to ovoid testes distributed transversely in straight line. External and internal seminal vesicles present. Cirrus 31 to 38 microns long, spinose. Ovary deeply lobed, not reticulate. Vitelline gland dorsal to ovary, irregular in shape. Enlarged vagina functioning as seminal receptacle in fully mature region. Early uterus a tubular structure, quickly differentiating into coarse network with occasional intersegmental connections. Mature eggs not seen.

Type host: Colymbus nigricollis

Habitat: Small intestine

Localities: Puerto Refugio, Guardian Angel Island, Gulf of California (Type); Salton Sea, Imperial County, California.

Type: U. S. Nat. Mus. Helm. Coll. No. 49089; Paratype No. 49090.

Parafimbriaria n. g.

Diagnosis: FIMBRIARIINAE without pseudoscolex. External segmentation lacking. Internal segmentation evident. Four longitudinal excretory ducts ventral to male and female genital ducts. Ovary lobed, not reticulate. Uterus initially a coarse network, not markedly metameric; later irregularly sacciform. Parasites of birds. Type species: *Parafimbriaria websteri.*

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* Present Address: Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

Discussion of *Parafimbriaria*

The strobilae of this genus are relatively large. The type specimen is 16 cm. long and measures 2.28 mm. in greatest width. However, since none of our specimens contained fully gravid uteri, the strobilae probably attain greater size. In relation to the remainder of the strobila, the scolex (Pl. 1, fig. f) and neck region are very slender and delicate so that the first 2 or 3 centimeters of the strobila have a wedge-shaped appearance. About 15 mm. posterior to the scolex the strobila broadens rapidly and is of about the same width through its remaining length (Pl. 2, figs. a, b, c). External segmentation is not evident in any part of the worm.

The mature reproductive systems are illustrated in Plate 2, figure b. Each male reproductive system consists of 3 ovoid to spherical testes, a slender vas deferens (vasa efferentia not observed), a prominent external seminal vesicle, and a cirrus pouch containing an internal seminal vesicle and a small cirrus armed with delicate spines. The female gonads are poral to the testes.

In the lack of both a pseudoscolex and a reticulate ovary and in having a reduced number of longitudinal excretory ducts, *Parafimbriaria* differs from all known species of FIMBRIARIINAE. Of these characteristics, the non-reticulate ovary seems to be the most outstanding. Among the genera referred to this subfamily (Webster, 1943) there is a gradation in the character of the pseudoscolex, ranging from a complete lack of this structure in *Parafimbriaria* to a well developed one in *Fimbriaria;* intermediate development of the pseudoscolex is seen in *Fimbriariella* and *Fimbriarioides*. It would seem that the presence or absence of a pseudoscolex may be of generic rather than of subfamilial significance. The same may be said of the number of longitudinal excretory ducts which range from 4 in *Parafimbriaria* to 11 in some species of *Fimbriarioides*.

Parafimbriaria differs from genera referred to the HYMENOLEPIDINAE in lacking external segmentation and in the ramified excretory system. Though in some respects, it shows greater affinity to hymenolepidinines than to other genera regarded as fimbriariine, we feel that at present *Parafimbriaria* must be regarded as a genus of the subfamily FIMBRIARIINAE.

Hymenolepis furcigera (Krabbe, 1869)

Joyeux and Baer (1950) record size data and some descriptive information on this cestode from grebes. They also place *Hymenolepis woodsholei* Fuhrmann, 1932, in synonymy with *H. furcigera*. *H. woodsholei* was the name given by Fuhrmann (1932) to the species described by Linton (1927) as *Hymenolepis podicipina*. Thus, Linton's description of this species is presumbably a description of *H. furcigera*.

In general, our specimens correspond to the existing descriptions of this species, but it seems desirable to present additional information. *H. furcigera* exhibits marked protandry. In proglottids with maximal testicular development, the ovary is not yet formed; only a small granular area marks its future position (Pl. 2, fig. d). At this stage, the seminal receptacle is a small, empty bladder, poral and adjacent to the ovarian primordium. The seminal receptacle opens into the relatively thick-walled vagina. A mature proglottid with testes at maximal size for this specimen is illustrated in Plate 2, figure d. At the proximal end of the cirrus pouch there is a prominent seminal vesicle which usually lies in the middle of the proglottid.

The female structures are as described. The cirrus is armed with fine spines. The cirrus pouch and the seminal vesicle have very thin walls. The 3 testes are, as a rule, in the middle portion of the proglottid, and their position is roughly triangular in most segments. More posteriorly in the strobila, the testes disintegrate and disappear, and ovarian maturation begins.

It should be noted that *H. furcigera,* at least as observed in our material, is a very delicate worm which appears as a fine thread to the unaided eye. The mature proglottid illustrated in our figure measured about 210 microns in width and about 35 microns in length.

Hymenolepis podicipina Szymánski, 1905

Before presenting descriptive notes on this species, it should be mentioned that the correct specific name is *podicipina* rather than *podicipedina,* as used by Joyeux and Baer (1936). In their study of hymenolepidids from grebes, the latter authors (1950) used the correct original name. Joyeux and Baer (1950) stated that *H. podicipina* was originally described from poorly preserved material, that no other descriptions of this species are available, and that in the original drawings the hooks resemble those of *Hymenolepis furcigera* (Krabbe, 1869) when seen from an unfavorable angle. Not having seen specimens of *H. podicipina,* Joyeux and Baer tentatively placed this species in synonymy with *H. furcigera,* acknowledging however, a difference in hook size between the two.

Since we fortunately obtained adequate material, we are able to present sufficiently detailed supplementary data on *H. podicipina* to establish the validity of this species. It should be emphasized that the shape of the hooks of this species is distinctive enough for accurate indentification. Of the many species of the genus *Hymenolepis, H. podicipina* is the only known species having this type of hook. The original drawing of Szymánski (1905) is illustrative of the hooks seen in our specimens. Hook number (10) and hook size in our material correspond to those described by Szymánski. In our specimens, the hooks are about 40 microns long, and in the original description the length was given as 42–46 microns. Scolex, sucker and rostellar diameters are somewhat smaller in our material, being about 250 microns, 86 microns, and 110 microns, respectively, as compared with corresponding measurements of 372 microns, 115 microns, and 150 microns presented by Szymánski (1905). The rostellum is a prominent, bulbous structure (Pl. 1, fig. e) and may be retracted into a muscular, rostellar sac. The musculature and excretory system in our material are as originally described.

Szymánski (1905) gave little information on the structure of the reproductive systems, but recorded the presence of complete protandry in this species indicating that at maximal testicular development the ovarian primordium is barely noticeable. Our observations confirm this; figures d, c, b, Plate 1 show consecutive stages in gonadal development. Figure d illustrates a proglottid in which the testes have attained maximal size. The ovarian primordium is seen as a medially situated group of darkly staining granules. Poral and adjacent to these is a small bladder, the seminal receptacle, which does not yet contain any sperm. At this stage, the vagina cannot be seen. The cirrus pouch is well developed and contains an internal seminal vesicle with sperm. The proximal portion of the vas deferens, which later functions as an external seminal vesicle, is not appreciably distended. A subsequent

stage in the development of the same specimen is illustrated in figure c. The testes have degenerated to about one third of their maximal size, and the seminal vesicles and receptacle are distended and filled with spermatozoa. The cirrus which is armed with fine spines is conspicuous within the cirrus pouch. The vagina is a considerably coiled and narrow tube. The appearance of the ovarian primordium has not changed in any way. Gradually, however, the remnants of the testes disappear in more posterior proglottids, and the ovary and vitelline gland begin to take shape. A proglottid in which the ovary has attained maximal size is illustrated in figure b. While the vitelline gland is irregular in shape and somewhat diffuse, the ovary consists of a few small and well circumscribed lobes. A definite connection is seen between the vitelline gland and the seminal receptacle which is filled with spermatozoa; porally, the seminal receptacle leads into the coiled and thin-walled vagina. Of the male reproductive system, the seminal vesicles and cirrus pouch are most prominent. Careful searching of such a proglottid may occasionally reveal small remnants of testes and vasa efferentia, as indicated in figure b by a dotted line.

The shape of the gravid uterus is as originally described by Szymánski (1905). Unfortunately, no fully gravid proglottids were seen in our specimens. A description of eggs and onchospheres is therefore omitted.

The present report is the first published North American record of what is unmistakably *H. podicipina*. The confusion regarding Linton's identification of *H. podicipina* has been discussed above in connection with *H. furcigera* Krabbe.

Hymenolepis recurvirostrae (Krabbe, 1869)

The finding of this cestode in *Recurvirostra americana* represents a new host record and is the first record of the worm from North America. Since previous descriptions of this species (Krabbe, 1869; Joyeux and Baer, 1936) do not include the anatomy of the female reproductive system, it seems desirable to present a description of these organ systems. An explanation for this deficiency in previous descriptions may lie in the fact that this tapeworm is characterized by extreme protandry. There are essentially three types of mature proglottids which can be conveniently designated as "testicular," "intermediate" and "ovarian" proglottids. In the "testicular" proglottids which are posterior to the immature region, the testes develop more rapidly than any of the other parts of the male reproductive system. A small, granular area on the ventral side of the worm marks the future position of the female gonads, but this area does not undergo further differentiation until the testes have completely disappeared. When the testes reach maximal size, the external and internal seminal vesicles (Pl. 1, fig. h), as well as the seminal receptacle are filled with spermatozoa. A connection between the vagina and the seminal receptacle can be seen in early "testicular" proglottids. More posteriorly, the vagina apparently atrophies. The testes are situated in a straight line and the genital pores are unilateral. After the complete disappearance of the testes, there is a variable number of "intermediate" proglottids containing the very prominent cirrus pouch with an internal seminal vesicle and cirrus, the external seminal vesicle, and the seminal receptacle. The cirrus may be extruded and is armed with minute spines. In these proglottids, no further development of female gonads is evident. The length of this intermediate area varies. In all specimens studied, there is evi-

dence of differentiation of the female gonads only after the testes have completely disappeared.

The "ovarian" proglottid (Pl. 1, fig. g) contains the lobed ovary and the coarsely granular vitelline gland in addition to the cirrus pouch, seminal vesicle and seminal receptacle. The ovary is in the middle of the proglottid. The vitelline gland is usually somewhat aporal and ventral to the ovary. The shape of the former varies in proglottids of the same specimen; it may be lobed, notched or smooth. The uterus first appears on the dorsal side of the proglottid; it gradually increases in size and eventually fills the entire proglottid (Pl. 1, fig. i) except for the median part where the uterus is usually constricted. Eggs are slightly ovoid or spherical and measure 22 to 24 by 29 to 31 microns. They consist of an outer, smooth shell which encloses the six-hooked onchosphere.

SUMMARY

Parafimbriaria websteri n. g., n. sp., is described from the grebe *Colymbus nigricollis;* relationships between this new genus and members of the FIMBRIARIINAE are discussed.

Additional descriptions are given of the following: *Hymenolepis furcigera* (Krabbe, 1869) and *Hymenolepis podicipina* Szymánski, 1905, both from the grebe, *Colymbus nigricollis;* and *Hymenolepis recurvirostrae* (Krabbe, 1869), from the avocet, *Recurvirostra americana.* With the exception of *H. furcigera,* these hymenolepidine species are reported for the first time from North America.

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EXPLANATION OF PLATES

All drawings made with the aid of a camera lucida.

PLATE I

FIG. a. *Parafimbriaria websteri;* rostellar hooks.

FIG. b. *Hymenolepis podicipina;* mature proglottid with female gonads and genital ducts; the testes have disintegrated.

FIG. c. *Hymenolepis podicipina;* mature proglottid with genital ducts, seminal vesicles and receptacle, and ovarian primordium; testes reduced in size.

FIG. d. *Hymenolepis podicipina;* mature proglottid with fully developed testes and cirrus pouch.

FIG. e. *Hymenolepis podicipina;* scolex.

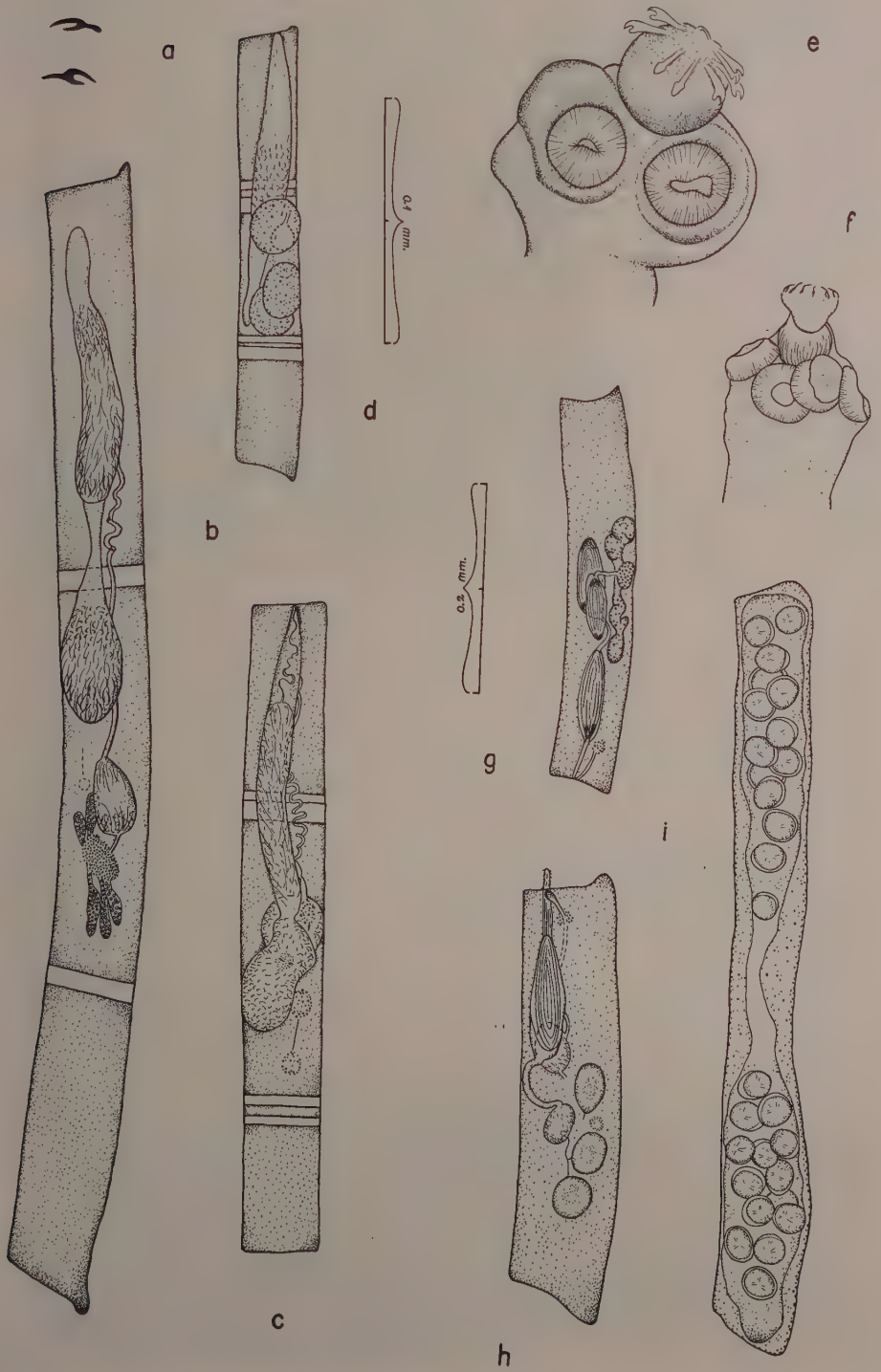
FIG. f. *Parafimbriaria websteri;* scolex.

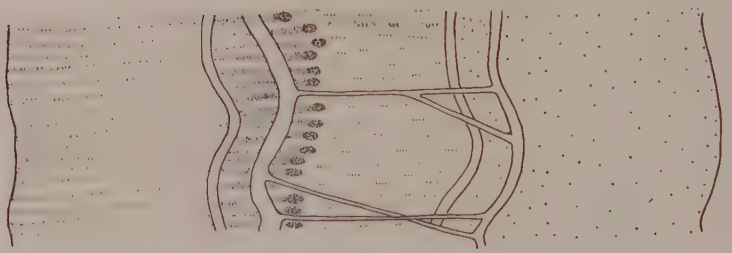
FIG. g. *Hymenolepis recurvirostrae;* mature proglottid with female gonads and genital ducts; the testes have disintegrated.

FIG. h. *Hymenolepis recurvirostrae;* mature proglottid with fully developed testes and ducts; small ovarian primordium present.

FIG. i. *Hymenolepis recurvirostrae;* gravid proglottid.

PLATE I





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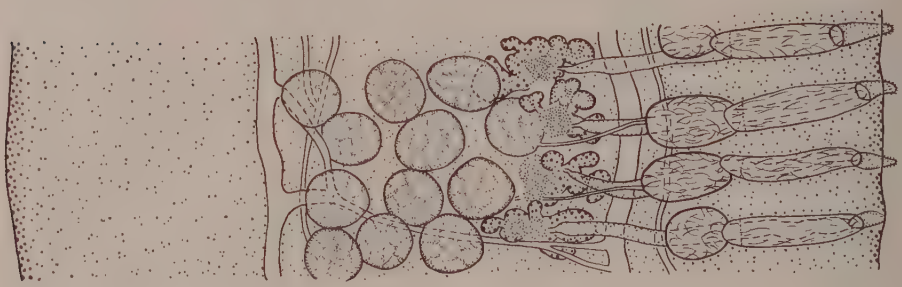

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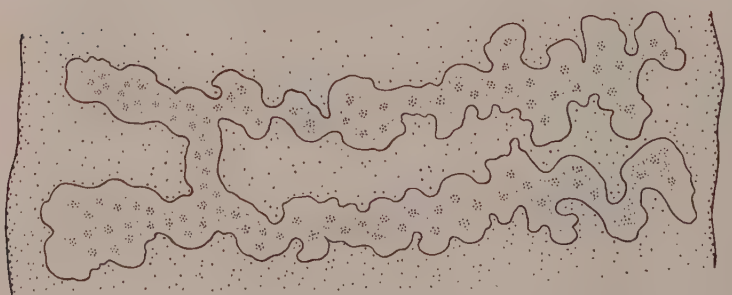

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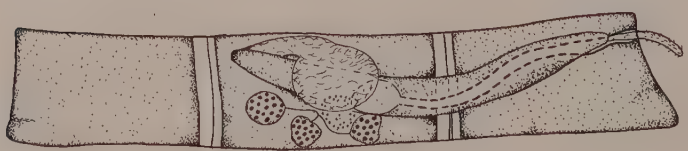

d

PLATE II

FIG. a. *Parafimbriaria websteri;* immature region with primordia of reproductive systems.
FIG. b. *Parafimbriaria websteri;* mature region.
FIG. c. *Parafimbriaria websteri;* two semi-gravid uteri.
FIG. d. *Hymenolepis furcigera;* mature proglottid with fully developed male reproductive system.

OBSERVATIONS ON *TRYPANOSOMA LEWISI* AND *GRAHAMELLA* SP. IN THE BLOOD OF RATS FROM THE HAMAKUA DISTRICT, ISLAND OF HAWAII[1]

LEO KARTMAN[2]

San Francisco Field Station, San Francisco 18, California

During the course of an investigation on wild rodent flea control in the enzootic plague region of the Island of Hawaii, observations were made on *Trypanosoma lewisi* in several species of rats inhabiting a wild gulch area of the Hamakua District. These observations were not the result of an organized study of blood parasites, since the original purpose of the investigation was to use *T. lewisi* as an indicator of the pulicidal effect of insecticides affecting rat fleas. It was thought that a significant reduction in the rat flea population would be reflected by a declining curve of new trypanosome infections in young susceptible rats. Unfortunately, the field investigations did not last long enough to allow an adequate evaluation of the above hypothesis.

A search of the literature has not revealed any reports on the blood protozoa of rats in the Hawaiian Islands. Furthermore, no evidence has been found on the incidence of blood protozoa in commensal rat species living in wild situations. Recent studies by Eyles (1952) and Calero (1952) review evidence on the incidence of *Trypanosoma lewisi* in rats from the American continent. Seven different studies cited by these authors, and their own investigations, show the incidence of *T. lewisi* in the Norway rat to vary from 0.5 to 47.5 percent. On the contrary, a study in Quebec, Canada, failed to demonstrate any blood protozoa in the brown rat (Firlotte, 1948). As far as can be determined, there is scant information on blood parasites of the black rat and its subspecies. Swellengrebel (1913) presented data on *T. lewisi* in *Rattus rattus rattus* in Eastern Java; all of his data were based on rats with a body length under 150 mm.

Doré (1918) found 30% of *R. norvegicus* from sewers and 12% from wharves and grain stores infected with *T. lewisi* in Auckland, New Zealand. This author also suggested that in New Zealand *Rattus exulans,* a member of the group to which the Hawaiian rat belongs, declined in numbers due to infection with *T. lewisi.* If this hypothesis is true it would substantiate the idea that *T. lewisi* is a relatively new parasite in rats of the Malaysian *concolor* group.

Laird (1951) obtained 65 *R. norvegicus* from buildings in Wellington, New Zealand and found 3 individuals infected with *T. lewisi.* The average dimensions of 100 trypanosomes recorded by this author are in substantial agreement with the mensural data given in the present report.

The present note will record observations on *Trypanosoma lewisi* mainly in the Hawaiian rat, *Rattus hawaiiensis,* in subspecies of the black rat, primarily *Rattus rattus alexandrinus,* and to a lesser extent in *R. norvegicus.*

Data also are presented here on an apparently unknown species of *Grahamella*

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[2] From the Communicable Disease Center, Department of Health, Education, and Welfare, Public Health Service, Atlanta, Georgia.

from the Hawaiian rat. The species of *Grahamella* occur within the erythrocytes of a variety of mammals and bear a superficial resemblance to *Bartonella*. The type species, *G. talpae*, was described from the blood of the European mole (Brumpt, 1911) in which the parasite originally had been discovered by Graham-Smith (1905). These parasites were formerly thought to be protozoa and were classed with the Anaplasmidae (Neitz, Alexander and Du Toit, 1934). The genus *Grahamella* now is thought to be a true bacterium (Tyzzer, 1942) and has been placed in the family BARTONELLACEAE (Bergey's Manual, 1948).

Several species of *Grahamella* have been described from rodents of the genera *Mus* and *Rattus*. These are: *G. muris* Carini from *R. norvegicus* in Brazil; *G. joyeuxii* Brumpt from *R. r. rattus* in French Guinea; *G. muris musculi iberica* Parwanidze from *M. musculus* in Transcaucasia; and *G. musculi* Benoit-Bazille from *M. musculus albinos* in France (Bergey's Manual, 1948). No reports have been found of *Grahamella* infections in the *exulans* type rats of the *Rattus concolor* group to which the Hawaiian rat belongs (Ellerman, 1941).

METHODS

A detailed account of methods and materials will be found in the report by Kartman and Lonergan (1953). For the present purpose it is sufficient to state that the rats were captured alive, anesthetized with ether or chloroform, marked by amputation of the toes, and released at the point of capture. Maturity of the rat was judged on the basis of testicular descent or vaginal perforation. Thin blood films were made from all individuals each time they were captured; the first film usually was from toe blood and subsequent films from tail blood. Two hundred fields of each Giemsa stained blood film were examined under oil immersion with 10× oculars and 97× objectives. Estimates of parasitemia were made in terms of the number of trypanosomes per 10,000 white blood cells. All blood films were obtained from rats inhabiting approximately 4,000 linear feet of Kaunamano Gulch at between 400 to 750 feet in elevation and located about 6 miles east of the town of Honokaa in the Hamakua District, Island of Hawaii.

DATA AND DISCUSSION

Trypanosoma lewisi:

The incidence of *Trypanosoma lewisi* in *Rattus hawaiiensis* was almost four times that in *R. norvegicus* and about twice that in *R. rattus* subspecies (Table 1). Of the 123 infected *R. hawaiiensis*, 26 had young infections (i.e., they showed actively dividing forms), whereas 3 of the 37 *R. rattus* infections were young and one of the *R. norvegicus* infections could be so classified. Of the *hawaiiensis* with young trypanosome infections, 42.8 percent were in juvenile rats, whereas 57.2 percent were in adults. All young infections in the other rat species were in juveniles.

Sex of the host appeared not to be a significant factor. In the *rattus* group 25 of 96 males, or 26.0 percent, were infected, whereas 12 of 70 females, or 17.1 percent, were infected. For the Hawaiian rat, 57 of 134 males, or 42.5 percent, were infected, whereas 66 of the 150 females, or 44.0 percent, were infected.

The possible relation of age to the infection rate was studied by an arbitrary division of the rats into weight classes (Table 1). For each species of rat, weights were divided into 6 classes which would include both upper and lower extremes

TABLE 1.—*Incidence of* Trypanosoma lewisi *in* Rattus *spp. from Kaunamano Gulch, Hamakua District, Island of Hawaii (July 1, 1952 to April 24, 1953)*

Weight class (grams)	*R. hawaiiensis*			Weight class (grams)	*R. rattus**			*R. norvegicus*		
	No. exam.	No. inf.	% inf.		No. exam.	No. inf.	% inf.	No. exam.	No. inf.	% inf.
0–14	1	1	100.0	0– 99	38	7	18.4	13	4	30.7
15–29	31	12	38.7	100–199	119	29	24.3	39	3	7.6
30–44	57	31	54.3	200–299	9	1	11.1	9	0	0.0
45–59	94	48	51.0	300–399	0	..	..	0	..	..
60–74	73	26	35.6	400–499	0	..	..	0	..	..
75–89	28	5	17.8	500–599	0	..	..	0	..	..
TOTALS	284	123	43.3		166	37	22.2	61	7	11.4

* Predominantly the subspecies *alexandrinus*.

hypothetically possible for the species. Individuals in all weight classes were found for the Hawaiian rat, whereas the commensal species included individuals falling into only 3 of the selected classes. The data indicate that all weight classes of the native rat had frequent infections although the most prevalent infections occurred in rats under 60 grams. For the commensal species the infection rate appeared to drop sharply in the individuals over 200 grams. This finding corroborates other studies (see Eyles, 1952). In general it should be noted that the commensal species attain sexual maturity when their body weight is about 75 to 100 grams, whereas the mature Hawaiian rat may be as low as 25 to 35 grams. Hawaiian rats weighing close to 90 grams are at their upper limit. No commensal rats over 300 grams were noted although they are known to attain weights of over 600 grams (Davis, 1949). These data suggest that, unlike the commensal species, there is little or no correlation between physical maturation and infection with *Trypanosoma lewisi* in the Hawaiian rat.

Differences also were found in the intensities of infection in individuals of the different rat species. In Figure 1 intensities of infection in Hawaiian and *Rattus*

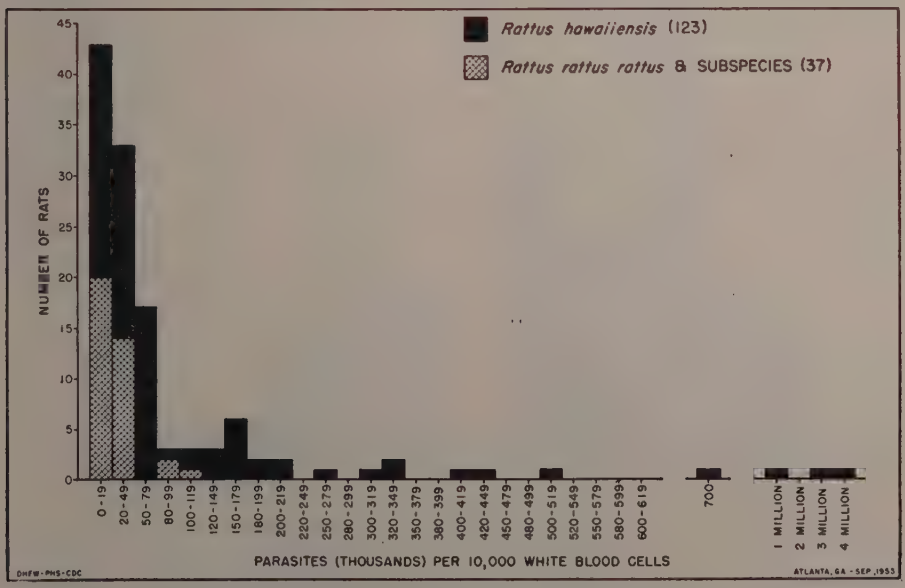

FIG. 1. Frequency of rats with varying intensities of *Trypanosoma lewisi* parasitemia in Kaunamano Gulch, Hamakua District, Island of Hawaii (number of infected rats in parenthesis).

rattus group rats are compared. The Norway rats have been omitted because too few individuals were captured. These data suggest that the parasitemia was more intense in the native rat than in the black rat and its subspecies.

Although circumstances prevented a study of infected rats in the laboratory, some data can be shown to indicate the duration of trypanosome parasitemias in individual rats captured more than once (Table 2). There are too few data upon which to base an analysis, but they suggest a more prolonged patency and a less precipitate decline in the *R. hawaiiensis* parasitemia than in the *R. rattus* group.

The foregoing data suggest that the relation between *Trypanosoma lewisi* and *Rattus hawaiiensis* may be a more recent one and less stabilized than in the commensal species. The higher infection rate, the greater intensity of infection, the apparent lack of a correlation between infection rate and age (weight), and the possibly longer patency of the parasitemia in the Hawaiian rat when compared with the commensal rats indicate that the well defined immunological relationships may not be established in the former host species as they are in the latter (see Taliaferro, 1924).

TABLE 2.—*Examples of patency of* Trypanosoma lewisi *in individual rats captured more than once*

Rat #	Rat species	Sex*	Date captured/Number trypanosomes per 10,000 wbc				
76	*hawaiiensis*	♂ j	8–7–52/0	9–16/196,666	9–25/44,285		
93	"	♀ j	8–21–52/326,666	9–16/120,000	9–18/96,000	9–23/48,000	
47	"	♂ a	7–17–52/0	8–6/15,000	8–13/8,000	8–14/3,000	
42	"	♂ a	12–16–52/50,000	1–13–53/55,000	2–17/0		
72	"	♂ a	12–31–52/17,700	1–28–53/14,000	2–26/300	3–6/900	4–1/0
152	"	♂ a	10–2–52/52,666	3–4–53/8,000	3–6/4,500	3–20/3,000	4–1/4,000
23	*rattus*	♀ j	7–9–52/11,888	7–17/8,461			
33	"	♀ j	7–10–52/7,307	8–28/3,947			
34	"	♂ j	7–16–52/0	7–30/4,375			
24	"	♂ a	12–10–52/37,132	12–11/37,142	2–10–53/7,400		
44	"	♂ a	7–17–52/24,210	8–27/3,617	9–18/0		
10	*norvegicus*	♀ j	12–5–52/15,000	2–17–53/3,000			

* j—juvenile. a—adult.

Although no exact records exist, it is probable that the commensal rats were introduced to the Hawaiian Islands during the 19th Century by the growth of world commerce (Spencer, 1938). The Hawaiian rat is considered indigenous and most likely colonized the islands during the early Polynesian migrations long before the advent of Western exploration in this region (Tate, 1935). This would lend support to the suggestion that *Trypanosoma lewisi* was introduced to Hawaii comparatively recently by the commensal rat species (probably *R. norvegicus*) and that the native rat may be a relatively new host for this parasite.

The Hawaiian rat flea, *Xenopsylla vexabilis hawaiiensis*, was the most prevalent flea species found on all species of rats in Kaunamano Gulch during the course of this study (Kartman and Lonergan, 1953). Midgut and hindgut smears of 7 fleas taken from infected rats were treated with Giemsa stain. Of these, 4 showed some attached crithidias, many free crithidial forms and some transitional forms. Although no typical metacyclic trypanosomes were noted in material from the hindgut of these fleas, this may have been due to the small number examined.

On the basis of epizootiological evidence, it appears probable that *Xenopsylla vexabilis hawaiiensis* is the principle intermediate host of *T. lewisi* in the particular area under consideration. Studies in other areas would probably show *X. cheopis* to be an important invertebrate host. A comparison of flea indices on the Hawaiian

rat with the trypanosome infection rate does not show a clear cut correlation but it does suggest that the incidence of trypanosome-infected rats was related to a rise or fall in the flea index (Figure 2). Changes in the flea indices are probably caused more by meteorological fluctuations, notably rainfall, than by other factors

FIG. 2. The monthly infection rates of *Trypanosoma lewisi* in, and the monthly indices of *Xenopsylla vexabilis hawaiiensis* on *Rattus hawaiiensis* from Kaunamano Gulch, Hamakua District, Island of Hawaii.

TABLE 3.—*Mensural data on 10 individuals of* Trypanosoma lewisi *in blood of individual rat hosts (mean values in microns)*

Rat host		Total length	Body width	Nuclear length	Nuclear width	Distance MN-A*	Flagellum
Species	No.						
hawaiiensis	20	32.1	1.8	2.6	1.4	8.7	7.6
*rattus***	20	30.0	1.1	2.3	1.3	8.3	7.3
norvegicus	7	31.2	1.9	2 1	1.1	8.8	7.4

* Middle of nucleus to anterior end.
** Predominantly the subspecies *alexandrinus*.

(Eskey, 1934; Kartman and Lonergan, 1953) and such influences are probably of major importance in flea-borne parasites of rats in the region.

Although *Trypanosoma lewisi* has not been reported heretofore from *Rattus hawaiiensis*, it is questionable whether the present form constitutes a subspecies even though it appears to have distinct physiological characteristics in the Hawaiian rat. The lack of host specificity alone would appear to negate such an hypothesis. Furthermore, the intense parasitemia and the characteristic location of the trypanosome nucleus near the anterior end would exclude it from the so-called *lewisi*-like forms. Mensural data (Table 3) on the parasite from the three species of rats in the area showed no significant differences and the values were well within the range of typical *T. lewisi* (Davis, 1952).

Grahamella sp.:

The erythrocytic parasites found in the blood of *Rattus hawaiiensis* have a morphology consistent with the genus *Grahamella* (Plate I).[3] No features were noted which could distinguish this form from grahamellae occurring in other host species. As seen in Giemsa stained, thin blood films, this organism consisted of fairly uniform rods which might be distributed throughout or in various parts of the erythrocyte, with occasional coccoid forms and very occasional groups of extra-erythrocytic rods. The latter are thought to be due to rupture of infected red cells in the process of preparing the blood film (Tyzzer, 1942).

An average of about 10 parasitized erythrocytes per 200 microscopic fields, and an average of about 17 parasites per infected erythrocyte was found. These data have been summarized in the form of frequency distributions and are presented in Table 4. Although erythrocytes containing large numbers of organisms

TABLE 4.—Grahamella *sp. in* Rattus hawaiiensis *from Kaunamano Gulch, Hamakua District, Island of Hawaii (July 1, 1952 to April 24, 1953)*

Host weight class (grams)	No. exam.	No. infected	Percent infected	No. parasitized rbc per 200 fields		No. parasites per rbc	
				Class	No. cases	Class	No. cases
0–14	1	0	0.0	0– 9	18	0– 4	2
15–29	31	1	3.2	10–19	6	5– 9	9
30–44	57	7	12.2	20–29	1	10–14	18
45–59	94	8	8.5	30–39	0	15–19	21
60–74	73	9	12.3	40–49	1	20–24	11
75–89	28	2	7.1	50–59	0	25–29	8
				60–69	1	30–34	3
						35–39	1
						40–44	1
						45–49	1
TOTALS	284	27	9.5	Range 1–60 Mean 10.8	27	Range 1–48 Mean 17.6	75

[3] This opinion was concurred in by Dr. David Weinman, Yale University, who examined a stained blood film.

were noted, none of these showed the intensity of infection characteristic of *Grahamella phyllotidis* of the Peruvian mouse (Tyzzer, 1942). It is possible that more heavily parasitized red cells might have been found if additional numbers had been examined.

It is notable that this parasite was not found in the commensal rats inhabiting the same region. However, it is not known whether the infection is present in the mouse, *Mus musculus,* which was very prevalent in the area studied, nor whether it may be present in commensal rats living in other areas of the Hawaiian chain. Furthermore, since splenectomy was not performed, it is not known whether this procedure might have elicited grahamellae in the commensal rats although splenectomy does not usually have any marked effect on *Grahamella.* Thus no definite statement can be made regarding host specificity except that the parasite seemed to be restricted to *Rattus hawaiiensis* in the area studied.

The incidence of infection in *R. hawaiiensis* is shown in Table 4. No obvious relation between age (weight) of the host and the infection rate was noted. No fluctuations suggestive of a seasonal trend were observed, the percentage infection rates by months being as follows: July 1952, 25.0; August, 36.3; September 52.3; October, 38.0; (November, no data); December, 55.2; January 1953, 42.5; February, 64.8; March, 36.0; and April, 34.7.

It is of interest to note that 12, or 44.5 percent of the infected rats also harbored *Trypanosoma lewisi* infections, whereas 15, or 55.5 percent of the infected rats showed no concurrent trypanosome parasitemia. Thus the phenomenon of "interference" appears not to be likely as in the case where both organisms are erythrocytic parasites (Tyzzer, 1941). The data suggest that sex of the host may have some influence upon the infection rate. In this connection, 9, or 33.3 percent of the infected rats were males, whereas there were 18, or 66.7 percent infected females.

It is believed that the *Grahamella* from the Hawaiian rat may be a new species. Nevertheless sufficient evidence is lacking to form the basis for a critical specific determination. As suggested by Tyzzer (1942), the criteria for the recognition of species of *Grahamella* and other related erythrocytic parasites are: (1) host specificity; (2) morphology; (3) pathogenicity; and (4) cultural characteristics. Since no distinctive morphology or pathogenicity are peculiar to the known species of *Grahamella,* species differences are based primarily upon Tyzzer's points 1 and 4. Although the organism from *Rattus hawaiiensis* appears to have a high degree of host specificity this point needs further elucidation as suggested above. Furthermore, its cultural characteristics are not known. For these reasons it would appear best to withhold a final decision regarding the specific status of the *Grahamella* from the Hawaiian rat until additional information becomes available.

SUMMARY

Observations are recorded in regard to infection of rats with *Trypanosoma lewisi* and *Grahamella* sp. in Kaunamano Gulch, Hamakua District, Island of Hawaii. The incidence of *T. lewisi* in *Rattus hawaiiensis* was almost 4 times that in *R. norvegicus* and about 2 times as great as that in *R. rattus* and its subspecies. Age of the rat (as judged by body weight) appeared not to be correlated with the trypanosome infection rate in the Hawaiian rat, whereas such a relation was present

in the commensal species. Parasitemia in the Hawaiian rat also appeared more intense and of possibly longer patency than in the commensal species. Thus it is suggested that the relation between *T. lewisi* and *R. hawaiiensis* may be historically more recent than in the commensal rat hosts. On the basis of epizootiological evidence it is suggested that the rat flea, *Xenopsylla vexabilis hawaiiensis*, is the principle intermediate host of *T. lewisi* in the particular area considered.

A species of *Grahamella* was found exclusively in *Rattus hawaiiensis*. No relation of age of the host to the infection rate was noted, nor were there any significant trends in the percentage infection rates by months. The data suggested that sex of the host may be a factor since twice as many females were infected as males. No "interference" between parasites was noted in rats harboring both trypanosomes and grahamellae. Although it is believed that the *Grahamella* from the Hawaiian rat may constitute a new species, reasons are given for withholding a decision on its specific status at this time.

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PLATE I

EXPLANATION OF PLATE

Grahamella sp. in blood of *Rattus hawaiiensis:* A—two erythrocytes with few grahamellae; B—two heavily parasitized erythrocytes with an adjacent polymorphonuclear leucocyte; C—grahamellae free in the blood; D—concurrent infection with grahamellae and trypanosomes.

LARVAL DEVELOPMENT OF *DIROFILARIA IMMITIS* (LEIDY) IN FLEAS[1]

EDMUND B. STUEBEN[2]

In most instances the transmission of this worm by any particular vector is correlated with the ability of the prelarval[3] microfilaria to develop to the infective stage. The theory of mosquito transmission of canine filariasis has been considered so firmly established since Grassi and Calandruccio (1890) observed developmental forms of *Dirofilaria immitis* larvae in *Anopheles maculipennis*, that the search for other arthropod vectors has been largely neglected. Laboratory tests have demonstrated that more than 26 species of mosquitoes have been experimentally infected with *D. immitis* larvae.

Susceptible mosquitoes have a high mortality rate when they are infected with *D. immitis* larvae. Unlike the larvae of other filariae such as *Wuchereria bancrofti* and *W. malayi*, which metamorphose among the thoracic muscles of the mosquito, the larvae of *D. immitis* undergoes the early stages of development in the malpighian tubules of the mosquito. In the course of this development the malpighian tubules of the mosquito are subjected to extensive damage so that it is improbable that they are still functional. Mortality rates as high as 100% due to this damage were recorded by Bradley (1953) for some species of mosquitoes.

The species of mosquitoes chiefly responsible for transmission of *D. immitis* in nature are not known.

During the latter part of the 19th century many workers in various countries, particularly Lewis (1875), Manson (1877), Grassi (1888), and Sonsino (1888) tried to find the intermediate host for *D. immitis*. Sonsino (1888) reported finding some *D. immitis* larvae not only in the flea (*Pulex serraticeps*), but also in the louse (*Haematopinus piliferus*). Bancroft (1901) reported negative findings for *Pulex serraticeps*. Breinl (1921) experimented with *Ctenocephalides felis* and *C. canis* and stated that the metamorphosis of the larvae took place in the malpighian tubules of the flea. Phillips (1939) declared negative findings with regard to *D. immitis* larvae in fleas and, therefore, concluded that fleas were not important intermediate hosts.

Summers (1940) reported finding both prelarval and developing larvae in the abdominal haemocoeles of *Ctenocephalides felis* and *C. canis*. On no occasion did he find any in the malpighian tubules as reported by Breinl. Bradley (1952) reported that the cat flea, *Ctenocephalides felis* was susceptible to *D. immitis* only under certain conditions.

It can be seen, therefore, that there was no concurrence of opinion as to the susceptibility of fleas to *D. immitis* or their ability to carry the worms to the infective stage.

MATERIAL AND METHODS

From January, 1951 through July, 1953, fleas were collected from 71 of the

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[1] Conducted at the University of Florida, Gainesville, Florida.

[2] The writer wishes to express his gratitude to Dr. Lewis Berner under whose direction this work was carried out.

[3] The term prelarval refers to that stage in the life history of the worm prior to its first molt.

142 dogs examined during this investigation. Since *Ctenocephalides felis* was found to be the most prevalent flea on dogs in central Florida, collecting, separation of sexes, and dissecting was concentrated on this species. The other species of fleas occasionally found on dogs were collected and colonies maintained in the laboratory for rearing and additional experiments. The species used were as follows: *Ctenocephalides canis, Xenopsylla cheopis, Pulex irritans, Echnidophaga gallinacea,* and *Orchopeas wickhami.* The classification is that of Fox (1940).

Of the 1203 *Ctenocephalides felis* collected and dissected, 342 were males and 861 were females. Many male fleas were observed on the dogs from which specimens were taken; however, the males were more elusive than the large, eggladen females. Advanced larval development of *D. immitis* was found in 111 males and in 335 females. All advanced "sausage stage" larvae, pre-infective and infective stage larvae were found in the haemocoele of the abdomen and thorax of these fleas.

In order to determine the percentage of infection of fleas feeding on an infected host an experiment was set up in which the fleas were restricted to the back of an infected dog. Fourteen days later 40 of the fleas were removed, sexed, dissected and examined for larval forms. Thirty-eight of the fleas showed evidence of infection. In many fleas microfilariae from recent blood meals were present as well as all stages of developing larvae ingested as microfilariae during preceding blood meals.

Metamorphosis of Dirofilaria immitis *larva*

A number of experiments were conducted to determine the time required for the larval metamorphosis from the prelarval form of the microfilaria to the infective stage. To do this a series of laboratory-reared infection-free adults of *Ctenocephalides felis, C. canis, Xenopsylla cheopis, Echidnophaga gallinacea, Pulex irritans,* and several *Orchopeas wickhami* were allowed to feed on an infected dog that, previous to the experiment, had been cleared of all ectoparasites.

During the feeding the fleas were restricted in large test tubes and fed through a 40 by 40 mesh per inch bronze screen. The host dog was tied down on a rabbit board and given a mild anesthesia. Under these conditions the fleas were able to feed for extended periods of time on the shaved abdomen of the dog.

When feeding ceased, the fleas were placed in gallon jars containing a sheet of paper toweling to absorb excretory wastes and capped with fine gauze to prevent the fleas from escaping. The containers were then stored in a constant temperature and humidity cabinet.

Young rats in restraining cages were placed in the jars to permit continued feeding. A dissection schedule was then set up for all of the fleas that had fed on the definitive host.

During the first 6 hours after taking a blood meal, samples were taken of all genera and species of fleas involved as well as of both sexes and dissected at 30 minute intervals. The following 6 dissections were conducted at 1 hour intervals. Thereafter the remaining dissections were performed at 12 hour intervals.

More than 1100 laboratory-reared fleas were dissected at set time intervals after they had fed on an infected host. In this way it was possible to plot the time, form of the developing larva, and its location in the intermediate host.

Dissections carried out during the desired time intervals required the use of 12 fleas (6 species with a male and female of each). As there were 32 scheduled

periods, a total of 384 fleas was required. When the first of 3 sets of dissections was made, more than the required 384 fleas were available and so were used. In repeating the experiment for the second and third times as a check against initial findings, only the more prevalent species (*Ctenocephalides felis, C. canis,* and *Xenopsylla cheopis*) were reared in sufficient numbers for all of the scheduled dissections.

Dissection Procedure

A flea that had fed on the infected definitive host was lightly anesthetized by ether and then placed in a drop of physiological saline solution on a slide. The flea was then dissected with a series of minute scalpels fashioned from No. 4 insect pins that had been heat tempered and finely honed. The primary dissections such as removal of head and opening the abdomen were performed under a magnification of 67.5 power. The dissections of the organ systems were made with a magnification of 112 power.

In fleas suspected of harboring advanced stages of larval development, the mouthparts were manipulated and examined prior to removal of head. Next the head was severed where it joined the prothorax and moved aside in the saline. The ventral surface of the thorax and abdomen were then opened and checked for larval forms in the haemocoele. The reproductive organs were then removed and carefully separated and examined. Finally, the complete alimentary tract was dissected out except a short section of the esophagus and the entire pharynx which were included with the severed head. The alimentary tract was carefully examined for perforations which would present easy exit from the midgut into the haemocoele for some of the weaker prelarval forms.

Observations

Metamorphosis of the larva

The metamorphosis of *D. immitis* microfilaria in *Ctenocephalides felis, C. canis, Xenopsylla cheopis, Pulex irritans, Echidnophaga gallinacea,* and *Orchopeas wickhami* at 27° to 29° C. shows the typical morphological changes that occur in the development of all filarial larvae as described by Connal and Connal (1922) for *Loa loa* larvae in *Chrysops silacea,* Blacklock (1926) for *Onchocerca volvulus* larvae in *Simulium damnosum,* and Yamada (1927) for *Wuchereria bancrofti* larvae in *Culex pipiens.*

The microfilaria of *D. immitis* is a sheathless form. In the course of the dissections it was noted that some of these prelarval forms had penetrated the midgut and entered the ventral region of the abdominal haemocoele as early as 1 hour after having been ingested with a blood meal by the flea.

Fleas dissected 2, 3, 4, and 5 hours after a blood meal showed a progressive increase in the number of prelarval forms located in the haemocoele in contrast to the numbers still active and free in the midgut.

Dissection of more than 300 infected fleas showed the earliest penetration of microfilariae into the abdominal haemocoele 1 hour after ingestion of the microfilariae. The greatest number penetrated within 4 to 5 hours after being ingested. This finding led to the conclusion that in the remaining 264 required dissections to complete the experiment, all larval forms would be found outside the midgut.

The development of *D. immitis* larva and its movements in the body of the flea are summarized in Table 1.

Effects of larvae on fleas

In order to be able to estimate the mortality rate of infected fleas as compared to infection-free fleas, 11 colonies of fleas were separated into 3 experimental groups. Groups A and B were the colonies of infected fleas and Group C consisted of 3 infection-free colonies. Group A consisted of 6 colonies of laboratory-reared fleas which took their first blood meal on an infected dog. Subsequent feedings by Group A were made on young rats confined in small cages to restrict movement. Group B consisted of 2 colonies of wild collected fleas taken from infected dogs. These were placed on young rats for further feedings. Group C was 3 laboratory-reared, infection-free colonies used as a control. They took all blood meals from young rats. In each of the 11 colonies composing groups A, B, and C a minimum

TABLE 1.—*Development of* D. immitis *larva in fleas*[a]

Time	Form of larva	Location in flea	
Blood meal plus			
30 min.	distinct	midgut	
1 hr.	prelarval	.....	
1.5 hrs.	stages		
2 hrs.			invading the
2.5 hrs.			stomach wall[b]
3 hrs.			
3.5 hrs.		in ventral	
4 hrs.		abdominal	
4.5 hrs.		haemocoele	
5 hrs.			
5.5 hrs.	internal structures		
6 hrs.	becoming more		
7 hrs.	distinct during		
8 hrs.	transition from	throughout the	
9 hrs.	prelarval to early	haemocoele of	
10 hrs.	larval stage	thorax and	
11 hrs.		abdomen	
12 hrs.	first larval stage		
24 hrs.	with a distinct,		
36 hrs.	thin tail		
48 hrs.	characteristic sausage	haemocoele	
60 hrs.	form of 2nd stage larva	of abdomen	
72 hrs.	preinfective form 3rd		
84 hrs.	stage larva		
96 hrs.			
108 hrs.			
120 hrs.			
132 hrs.			
144 hrs.			
158 hrs.	mature infective	majority in the	
168 hrs.	stage larva	haemocoele of the head	
180 hrs.		and thorax. Some in abdominal haemocoele	

[a] *Ctenocephalides felis, C. canis, Xenopsylla cheopis, Pulex irritans, Echidnophaga gallinacea, Orchopeas wickhami.*

[b] Dotted line indicates time of penetration of stomach wall by prelarval forms.

of 12 males and 12 female fleas were placed on ectoparasite-free young rats. Observations on anesthetized fleas and sample dissections of the balance of fleas from Groups A and B were made and their infection verified by the presence of developing larvae of *D. immitis.*

Daily observations made over a twenty-day period showed no difference as to mortality rate of the infected fleas of Groups A and B as compared to the infection-free fleas of control Group C.

Flea eggs deposited by females of Groups A and B as well as of Group C were noted. The counting of flea eggs in all 11 colonies comprising the 3 groups was not considered because of two factors: (1) the question of viability of the eggs,

and (2) the time required for an accurate count of the enormous number produced. The problem that was presented was solved rather simply by waiting until the eggs hatched and the flea larvae went into the pupa stage. After sifting the pupae out of the sand and debris their volumes were measured in a graduated tube. The volumes of pupae of the control Group C were not larger than the volumes of pupae from the infected colonies comprising Groups A and B. The pupae of all colonies were kept for emergence of adults; here again there was no apparent difference as to numbers of adults.

The results of these experiments clearly show that there is no increase in the mortality rate nor apparent effect on the fecundity of the female fleas or fertility of the male fleas harboring an infection of *D. immitis* larvae.

Activity of larvae in the flea

As shown earlier in Table 1, the majority of the infective stage larvae were found in the haemocoele of the head and thorax of the dissected infected fleas. These infective stage larvae, because of their high degree of motility, were able to move against the haemolymph flow and leave the crowded, organ-packed haemocoele of the abdomen.

In order to observe the movements of the infective stage larvae in the living flea, fleas were placed in several restraining media such as polyvinyl alcohol and methyl cellulose solutions. These were not effective in restricting the movements of the active flea; therefore, ether was employed to anesthetize the insects.

The lightly anesthetized flea was then placed on a glass slide and the internal regions of the head, thorax, and abdomen were observed with the aid of 100 and 440 magnifications. In each case a strong beam of transmitted light rather than reflected light was used as a light source.

In almost all cases the activity of the filaria larvae could be observed in the flea. The greatest difficulty was encountered when some of the larvae moved into the abdominal haemocoele. The crowded organs were best seen being pushed about in the lightly chitinized *Xenopsylla cheopis* as the active larvae moved among them. The larvae were visible at times; however, when they stopped their movement it was impossible to distinguish them from the internal organs of the flea.

The movement of the larvae in the haemocoele of the thorax and abdomen can best be described as being that of a typical nematode. In the haemocoele of the head, however, they displayed more of a gliding action with a minimum of lateral motion. The speculation arose as to why the decrease of activity in the haemocoele of the head was such a contrast to the rather violent motion exhibited in the thorax and abdomen. Then it was noticed that one of the larvae which had apparently penetrated, in part, the minute haemocoele of the labrum suddenly moved backwards. The gliding movement exhibited by the larva in the haemocoele of the head resulted from the restricted area for movement.

The backward movement of the larvae from the head into the thorax was induced by gently applying an ice cold blunt dissecting pin to the mouthparts of the flea. In other tests a drop of saline was put over the mouthparts of the anesthetized flea and shortly thereafter could be observed the forward movement of the larvae wriggling in the abdomen, subdued activity in the thorax, and then the restricted movement into the head.

Method of escape of the larva from the flea

The mechanism of the escape of the infective stage larva from the intermediate to the definitive host follows a very intricate pattern. The first requirement is that the fully developed larva be located in the vicinity of the mouthparts.

Menon and Ramamurti (1941) in their investigation of *Wuchereria bancrofti* larvae, found that with moderate warmth (about 35° C.) the infective larvae became very active and could be observed moving to the very tip of the proboscis of infected *Culex fatigans*. They suggested that warmth was a definite factor in larval escape since it brought about increased activity.

My experiment with the mature larvae of *D. immitis* in various infected fleas also showed a similar pattern of larval concentration in the head region when warm physiological saline (36° C.) was placed on the mouthparts of the flea.

After having succeeded in concentrating the larvae in the haemocoele of the head, a series of stimuli were tried to induce them to leave the body by moving out of the mouthparts. The first test involved use of hot saline (45°–52° C.) around the mouthparts. With this, those that did not die in place rapidly moved back into the haemocoele of the thorax and abdomen. Cool saline (22°–24° C.) did not kill any of the larvae; however, all left the near vicinity of the head and withdrew into the body.

Warm lymph (36° C.) and later warm blood (36° C.) increased the activity of the larvae in that they moved from the haemocele of the abdomen and thorax into the haemocoele of the head. However, this increased activity was no greater than that brought about by using the warm saline. The results of the test with cold lymph and cold blood were the same as those with the cold saline.

These simple tests showed that the chemical stimulus was incidental. The primary stimulus proved to be the presence of a warm fluid over the mouthparts, for this alone increased the concentration and activity of the larvae in the haemocoele of the head. No escape of the infective stage larva was seen after the external application of warm fluids to the mouthparts.

Attempts to section serially the infected fleas in the act of feeding were not successful.

As mentioned earlier, infective stage larvae were observed making partial entry into the haemocoele of the labrum, the lateral walls of which are membranous. Infective stage larvae were also observed in the vicinity of the labium and what appeared to be a partial entry into its haemocoele. During the course of this investigation there were 14 cases where manipulation of the mouthparts resulted in the emergence of infective stage larvae from the mouthparts of the flea when this region was immersed in warm saline and slight pressure was applied to the abdomen by placing a coverslip on it to stimulate distention.

On many other occasions when an engorged flea containing infective stage larvae was injured in any way by improper handling, it was not unusual for the infective stage larvae to emerge by way of these injured areas aided in part by the high intracoelomic pressure brought about by the blood meal. Summers (1943) reported that in *Pulex irritans* "Mature larvae [*D. immitis*] were seen penetrating through the membranes which connect the chitinous plates composing the body wall of the flea."

Many of the present day texts have statements like this: "They (the infective

larvae of filariae) are not directly inoculated by the bite [referring to the blood feeding arthropod] but pierce the skin, enter the circulations" (Stitt, Clough & Clough, 1945).

Such statements as the quotation above from the text were assumed to be true, but when examinations were made of serial sections of skin which had been pierced by arthropods infected with several infective stage larvae, the findings were meager. Serial sections of the skin of hosts showed no evidence of larval penetration after an injured flea from which infective stage larvae were emerging was placed on the skin.

Since nothing was known as to the speed of penetration and where the larvae moved once they entered the definitive host, it was decided to attempt to count the number of infective stage larvae in the flea before and after a blood meal. This, of course, was not accurate. However, by gross examination, as previously described, it was possible to estimate whether one or more infective stage larvae were present. In this experiment only fleas in which infective stage larvae could be seen were used. The skin of a new-born rat upon which these infected fleas were fed was removed, fixed, sectioned serially, stained, and checked for the presence and number of infective larvae. The infected flea that had taken the blood meal was then dissected and examined for the presence and number of infective larvae.

TABLE 2.—*Test of capability of larva to penetrate intact skin*

No. of larvae	Intact skin	Time	Results
6	Shaved leg of dog	1–15 min.	no penetration, died*
9	Back of young rat	1–15 min.	" " "
3	Belly of young rat	1–15 min.	" " "
4	Ear of dog	1–15 min.	" " "
2	Finger of man	1–15 min.	" " "

* Died because of dessication.

The majority of skin samples did not show the presence of larvae. At the same time, dissection of these infected fleas that had taken a blood meal on the skin showed a frequent decrease or complete absence of all previously observed infective stage larvae.

In the course of this experiment 47 infective stage larvae were observed in 28 infected fleas; the number of larvae was, in all probability, higher. Three skin samples were positive for larvae. Dissection of the 28 fed infected fleas showed that 17 infective larvae remained in these fleas.

Probably more than the 27 infective stage larvae discharged from the infected fleas were not accounted for. The statement that larvae were capable of penetrating skin became questionable and therefore a series of experiments were set up to determine whether it was true.

A series of infected fleas, *Ctenocephalides felis, C. canis* and several surviving infected mosquitoes, *Psorphora ferox,* were dissected in saline as a source of infective stage larvae. The larvae were then recovered in micropipettes and placed on various types of intact skin in a drop of saline or lymph and observed through a dissecting microscope. The results are summarized in Table 2.

The next test was with skin that had been broken by using insect pins, dissecting needles, and a sharp pointed scalpel to simulate the puncture made by an actively feeding flea. The openings lacked the anticoagulin that the feeding flea pours into the wound with its saliva. Heparin (1:9000) and also saline were applied; how-

ever, this added to the difficulty of temperature regulation, dropping it to such a point that the larvae, sensitive to slight temperaure changes and postively thermotropic, would not move toward the wound. Because of these factors and repeated failures, the addition of dilutants and anticoagulants was discontinued. When the larvae were dropped over the puncture in the skin, penetration into it was rapid; however, those near the edges of the puncture just wriggled back and forth until they dried up.

From the information thus gained it may be assumed that when the infective stage larvae escape from their intermediate host, whether through the mouthparts or through the broken body wall caused by the crushing of the flea by the host, the larvae might penetrate the definitive host under these conditions and in this sequence: 1) emerge from the flea into a moist medium that prevents their dehydration; 2) that warm blood or tissue fluid is exuding from the wound; 3) the distance between the wound and the larva is bridged with a moist medium; 4) that this distance be less than 2 millimeters.

SUMMARY AND CONCLUSIONS

A study of the relationships existing between the dog heartworm, *Dirofilaria immitis*, and various species of fleas was undertaken in order to determine the effects of the parasite on a potential intermediate host. The findings and conclusions resulting from this study are summarized below:

1. 446 of the 1203 *Ctenocephalides felis* collected from 71 dogs were infected with *D. immitis* larvae in advanced stages of development.
2. When specimens of *Ctenocephalides felis* and *C. canis* were restricted to feeding on an infected dog, 98% of them became infected with *D. immitis*.
3. The development of *D. immitis* microfilariae to the infective stage was traced in both males and females of *Ctenocephalides felis, C. canis, Xenopsylla cheopis, Pulex irritans, Echidnophaga gallinacea,* and *Orchopeas wickhami*.
4. The prelarval forms of *D. immitis* penetrated the midgut wall and entered the abdominal haemocoele of the flea as early as 1 hour after having been ingested with a blood meal.
5. The first and second stages of development of *D. immitis* larvae take place in the haemocoele of the abdomen.
6. The third stage larvae were found in the haemocoele of the abdomen and thorax.
7. The majority of the infective stage larvae were found in the haemocoele of the head and thorax.
8. Dissections and serial sections of infected fleas showed that no major tissue damage or apparent injury of any organs could be attributed to the developing larvae.
9. The fertility of the male fleas was not affected in any way by the developing larvae, nor was the egg production of the female fleas changed by the larvae infecting them.
10. There was no apparent increase in the mortality rate of infected fleas as compared to that of non-infected fleas.
11. The infective stage larvae were shown to be thermotropic, concentrating in the haemocoele of the head of the flea when warm saline was placed in its mouthparts.

12. The infective stage larvae are apparently incapable of moving out of an unfed flea.

13. In 14 cases infective stage larvae escaped through the region of the mouthparts of fed fleas when the intracoelomic pressure of the flea was increased.

14. Infective stage larvae were observed leaving the body of the fleas through injured areas of its exoskeleton.

15. Infective stage larvae proved to be incapable of penetrating intact skin.

16. Prior to feeding, 47 infective stage larvae were observed in 28 infected fleas. After feeding, the fleas were dissected and revealed only 17 remaining infective stage larvae. Skin samples taken from the areas bitten by the fleas showed the presence of 3 *D. immitis* larvae in the punctures caused by the feeding of the fleas. This demonstrates that infective stage larvae are capable of leaving the flea and entering the skin puncture made by it.

17. Because of the high percentage of infected fleas found under natural conditions, and because of the close association between dogs and fleas, these insects are most likely the natural vectors of canine filariasis.

18. It is very apparent that filarial transmission is far less efficient than the transmission of malaria where every bite results in a positive inoculation. Under these conditions the rate of infection of the intermediate host can be high and the rate in the definitive host low.

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DIETHYLCARBAMAZINE (HETRAZAN®) IN THE TREATMENT OF STRONGYLOIDIASIS

ELI CHERNIN[1]

Department of Tropical Public Health, Harvard School of Public Health, Boston

Although gentian violet is usually cited as the drug of choice in the treatment of strongyloidiasis its use has not been attended with uniform success. Therefore, despite reports that Hetrazan had proved ineffective in treating strongyloidiasis (Napier, 1949; Torres, 1950), it seemed worthwhile to re-assess its therapeutic possibilities under different circumstances and dosage levels. Such a study was undertaken upon the completion of a survey (Chernin, 1954) of intestinal parasitic infections among 750 workers at the Ludlow Jute Company's mill at Chengail, Howrah District, West Bengal. The survey showed that 8.1 percent of the workers were infected with *Strongyloides stercoralis* and from among them 7 cases of varying severity were selected for treatment.

MATERIALS AND METHODS

The 7 mill workers (6 males and 1 female) ranged from 24 to 61 years in age and from 37 to 55 kgm. in body weight; all received 6 mgm. of Hetrazan per kgm. 3 times daily for 6 consecutive days. These persons continued in their regular work in the mill during this period but reported to the dispensary at 8:00 A.M., 2:00 P.M., and 6:30 P.M., daily for treatment. The drug was administered in powdered form mixed with water and the workers were under careful individual surveillance to insure that each dose was consumed in its entirety. Stool examinations were made prior to treatment on the first day and were continued thereafter for the 6 day period with additional examinations 2 and 9 days after the termination of treatment. Stools were collected in clean containers at the dispensary and examination of the material was begun within 20 minutes of passage. A single stool per day from each individual was examined by each of the following technics: a) 2 direct saline smears of fairly uniform density prepared from different samples of the fecal mass; b) a single zinc sulfate concentration specimen prepared by centrifugation; and c) 2 direct saline smears of similar density made from a 48-hour filterpaper culture. An additional procedure, used only in the examination of stools of the 9th post-treat-

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ment day, was a zinc sulfate concentration specimen prepared from the entire fecal culture of that day. All preparations were examined in their entirety, counts were made of all *S. stercoralis* larvae seen, and the results expressed as a daily total. Although there are obvious variables involved in the procedures used these counts serve as a crude quantitative gauge to changes in the status of the infection during the course of treatment.

RESULTS AND COMMENT

The ineffectiveness of the course of Hetrazan may be gathered from the fact that *S. stercoralis* larvae were absent from the stools of only 2 of the 7 cases on the 9th post-treatment day. However, the data for the group as a whole indicated that fewer larvae were being passed 9 days after treatment (when the individual total counts were 2, 0, 27, 2, 52, 0, 15) than on the day treatment was begun when the respective counts were 40, 18, 215, 22, 482, 9, 31. Evidences of toxicity, probably related to the large doses of Hetrazan, were observed in 5 of the 7 cases; weakness, nausea, and vomiting occurred in all 5 while one patient also complained of such severe epigastric pains that treatment was omitted on the 3rd day of the course. The parasitologic results of this study are in keeping with those of Torres (1950) who reported that only 1 of 12 cases in Puerto Ricans became negative for larvae of *S. stercoralis* after treatment with Hetrazan. A similar experience has since been reported from Brazil (Coutinho, Croce, Campos, and Amato Neto, 1952), although other workers have published more favorably on the use of Hetrazan in human and in canine strongyloidiasis (Sotolongo, 1950; Werner, 1949).

SUMMARY

Seven cases of strongyloidiasis among workers at a jute mill in West Bengal were treated with diethylcarbamazine (Hetrazan[R]) in doses of 6 mgm. per kgm. of body weight 3 times daily for 6 consecutive days. Nine days after the completion of this course the stools of only 2 of the 7 were negative for *S. stercoralis* larvae, although the treatment reduced the number of larvae in the group as a whole. Toxic manifestations such as nausea, vomiting, weakness, and epigastric pain were observed in 5 of the workers treated.

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SURVIVAL OF GORGODERINE TREMATODES IN EXPERIMENTALLY ALTERED ENVIRONMENTS

CHAUNCEY G. GOODCHILD[1, 2]

Department of Biology, Emory University, Emory University, Georgia

Host specificity is a well recognized concept in parasitology. Less well recognized, perhaps, is an organ specificity which operates in parasitic orientation to—and maintenance in—specific organs of the host.

In a series of papers on the bionomics of gorgoderine trematodes (cf. Goodchild, 1943, 1945, 1948, 1950) a seeming adaptive plasticity has been noted in which "bladder flukes" penetrated and often reached maturity in urinary bladders, in mesonephroi and mesonephric ducts, in the digestive tract of fishes, and even in the esophagus and gills of fishes. Migratory postmetacercarial juveniles have additionally been encountered in the stomach, intestine, cloaca, and oviducts (Goodchild, 1948). Since growth to sexual maturity may occur in non-vesical sites, experimental transplantation into new organs should clarify interesting biological and physical interactions between host and parasite.

MATERIALS AND METHODS

Gorgoderina attenuata from *Rana pipiens* purchased in Wisconsin and Minnesota, and *Gorgodera amplicava* from *R. catesbeiana* purchased in Louisiana and Missouri were homo- and hetero-transplanted into these hosts and into *R. sylvatica* purchased in North Carolina. Survival was determined after transplantation, and also after alterations of the normal *in vivo* habitat and variations in *in vitro* cultures. Transplantations of bladder flukes were attempted intra- and interspecifically into anuran bladders, into the conjunctival sac, anterior eye chamber, dorsal lymph space, vocal sac, lung, and coelom. Special apparatus was devised to measure oxygen consumption and to perfuse the urinary bladder with fluids and gases to ascertain effects of alteration of the normal environment. Preliminary tests were made to determine parasitic longevity in urine and in artificial saline solutions of varying content and concentration.

All operations were performed oligoseptically and under urethane or ether anaesthesia. Donor frogs were sacrificed by pithing, while recipient frogs were anaesthetized.

Bladder-to-bladder transplantation was accomplished by placing an anaesthetized frog on an inclined block with the rump up and the cloacal aperture dilated with a retractor improvised from a nasal speculum such as is used for examination of children. Insertion of flukes directly into the bladder with a mouth pipette was viewed with a dissecting microscope equipped with strong vertical illumination. At the termination of the experiment recipients were sacrificed and parasites recovered and examined for normality. Whole mounts stained with Semichon's acetocarmine were finally prepared.

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Transplantation to the eye involved placing flukes in the conjunctival sac and in the anterior chamber. In the former, parasites were inserted into the lower part of the conjunctival sac, air bubbles were carefully excluded, and the lower lid was pulled upward over the surface of the eye and stitched in place by two sutures (braided silk 4-0 atraumatic) through the upper lid. For intraocular insertion a tiny incision was made with a Bard-Parker No. 11 blade through the corneal limbus near the roof of the iris. A parasite was introduced in amphibian Ringer's solution, after which the incision was closed by a single suture made also with braided silk thread. Flukes were recovered and prepared as whole mounts after staining with Semichon's acetocarmine and also were sectioned *in situ* and stained with Harris' hematoxylin and eosin and also with Masson's trichrome stain.

Insertion into the dorsal lymph sac was made through an incison, in the mid-dorsal line, large enough to receive the end of a small mouth pipette. The fluke was inserted and the lips of the incision pinched together. Worms were recovered after varying periods of time, and were fixed and stained.

The technique of insertion into the vocal sac was similar to that used for the bladder. Mouths of male frogs were opened, vocal sac openings dilated and flukes inserted by mouth pipette. In some hosts the opening was subsequently plugged with cotton or cork trimmed to the proper shape. Worms after recovery were fixed and stained.

Lungs used as recipient organs were pulled to the outside through a lateral horizontal incision made just behind the forelegs. A tiny incision was made into the lung in a zone of minimum blood vessels and the fluke inserted. The lung was returned to the body cavity after hemorrhage had been checked and the incision was closed by small metal clamps. Flukes were also inserted into the lung through the glottis and trachea. Some lungs in both types of implants, were deprived of air either by ligature, at the base of the lung in the former, or by tracheal plug and glottal suture in the latter. At the termination of the experiment the frog was sacrificed, the flukes recovered, studied and then made into permanent mounts.

Coelomic implantation occurred through an incision made through the ventro-lateral abdominal body wall and the lateral edge of the rectus abdominis muscle. Clamps were used to close the incision. Flukes, recovered by sacrificing the host, were made into stained whole mounts and sections.

Alteration of the normal *in vivo* vesical environment was accomplished by means of liquids or gases passed through a narrow-bore polyethylene tube, supported and guided by an external jig, inserted through the cloaca into the bladder. To pre-prevent the toes from pulling the tube from the bladder, the hind legs were extended and taped together at the knees. Whole flukes or fragments were recovered from the container in which the frog was placed or were taken from the bladder after the host had been sacrificed.

In vitro cultures were maintained, aerobically or anaerobically, in small Stender dishes. Survival was determined in saline solutions of varying concentrations, in urine, and in saline solutions into which air and also nitrogen were bubbled.

Observations

Initial auto-, homo-, and hetero-transplantations into bladders were performed to determine whether artificial manipulations, both inside and outside the host, had

permanent ill effects on parasites. Several dozen *Rana sylvatica* had been found free of bladder flukes and were used in these early transplants. Nine recipient *R. sylvatica* had implanted into their bladders at least 1 each of *Gorgodera amplicava* from donor *R. catesbeiana;* 11 others received specimens of *Gorgoderina attenuata* from donor *R. pipiens.* From 1 to 21 days later recipients were sacrificed and all implanted flukes recovered. They were completely normal in behavior and morphology. Auto-, homo-, and hetero-transplantations between *Gorgodera amplicava* and *Gorgoderina attenuata* in *R. pipiens* and *R. catesbeiana* in a comparable series of experiments yielded identical results.

Whereas implants into bladders continued to live, those into the conjunctival sac and anterior chamber of the eye quickly died. A total of 15 *Gorgoderina attenuata* and *Gorgodera amplicava* placed in the conjunctival sacs of *R. pipiens* and *R. catesbeiana* lived approximately 30 to 40 hours after which they became incorporated in a mass of host cells (the process of encirclement and infiltration will be described in more detail for the intraocular implants). Within 48 to 72 hours no recognizable signs of life were evident. Deteriorative sloughing of cuticula and edema of the ventral sucker caused the latter to bulge from the ventral margin and the prosoma to bend dorsally at right angles, at the same point, from the hind body.

The cellular picture of host defense to implantation in the anterior chamber was more thoroughly studied. Seventy-three transplantations revealed a very prompt and consistent host response. Corneal transparency allowed gross inspection with a binocular dissecting microscope of the early post-operative reactions, while sectioned and stained material permitted cellular analysis. The operation was a bloodless one with any deflation of the cornea, through loss of aqueous humor, corrected within 12 to 15 hours. Single flukes were placed in the lower corneal-iris angle and the incision quickly closed with a single dorsal median suture. In a few instances, the outer surface was smeared with vaseline or celloidin to seal the wound and to exclude oxygen.

In every instance of intraocular implantation death quickly ensued. Flukes appeared from the beginning to be totally unable to cope with the new environment. They were ineffectual in grasping any intraocular surface and soon slumped into the lower corneo-iris angle. Usually within 12 hours motion had ceased; in only one case did a worm live for 22 hours, as evidenced by slight mobility in the prosoma, and this one, too, was considered dead at 24 hours. The cuticula of implants became wrinkled, the ventral sucker became distended, and a gradual opacity spread throughout the organism.

Within 24 to 36 hours the implant became embedded in a mass of leucocytes which made its clean removal difficult. Sectioned material revealed the source and nature of host cells involved in the attack and penetration of the parasite. Vascular engorgement and extravasation, from vessels in the outer margin of the iris, of basophiles, small monocytes, eosinophiles (1%), and a few lymphocytes were noted within 4 hours of implantation (Fig. 1). By this time, also, minute breaks were observed in the post-acetabular cuticula around which masses of leucocytes, often 10 to 20 or more cells thick, were clustered. Parenchymal invasion by leucocytes started before the fluke became immobile. Extravasated erythrocytes began to invest the parasite by 8 hours, while invasive leucocytes continued to penetrate and spread from cuticular fissures deeper into the parasite. Cellular integrity of

parasitic organs, however, was still good. Generally the side of the parasite touching the iris became histolysed and cellularly confluent with it, while the underlying pigment layer of the iris became diffuse and disorganized. Blood cells were adherent to the parasite or to the iris; they were not generally free in the aqueous humor.

Within 16 hours post-implantation the normal histological picture of the fluke had disappeared (Fig. 2). Cuticula, reproductive and digestive organs, and miracidia and egg shells had been successfully attacked and histolysed. The parenchymatous inter-organ matrix was replaced by a diffuse, granular myxomatous meshwork containing numerous host leucocytes. Surrounding the parasite was a continuum of this same network containing streaks of diapedetic leucocytes moving toward the fluke from engorged blood vessels on the surface of the entire iris. Oral and ventral suckers were the only organs with any semblance of normal histology. Cellular adhesion to the iris was continued and was augmented by a firm adherence to the cornea as well. Corneal capillaries subjacent to the parasite remained unaltered while those at the corneo-scleral junction became progressively engorged and tortuous. Leucocytes in and around the parasite were similar in kind to those previously seen except for eosinophiles which surprisingly were now completely absent.

Thirty-six hours after implantation the form and content of the parasite were only vaguely persistent, the former being externally confluent with myxomatous material nearly filling the lower half of the anterior chamber, and the latter being internally largely replaced by host phagocytes (Figs. 3 and 4). Even the dense musculature of oral and ventral suckers was being invaded and the lumina of the suckers were filled with leucocytes. The anterior chamber finally (after 48 hours) became occluded with the myxomatous ground substance and innumerable red and white cells (Fig. 5). This outflow of corpuscles was so copious that it was apparent through the nearly opaque cornea before fixation.

Although there were rare instances where this time schedule of attack, penetration and histolysis was delayed, in the main it was the one observed. Grossly at 96 hours, the eye was turgid and somewhat enlarged and the cornea was opaque. Internally the fluke was almost totally replaced by host phagocytes (Fig. 6).

Host responses to implants were similar both homo- and hetero-plastically with no striking alteration in the described pattern of corpuscular response or time of ultimate death.

Bladder flukes implanted homo- and hetero-plastically into the dorsal lymph space also were dead, generally within 24 hours and certainly within 48 hours, and were incorporated in a mass of host cells confluent with the inner surface of the integument. If the parasite was first enclosed in a securely tied, thin celloidin bag filled with 70% amphibian Ringer's or frog urine before implantation death could be slightly delayed. Some flukes were found clinging to the bag 24 hours post-implantation; these were invariably dead, with the usual dorsal prosomal flexure and acetabular hypertrophy, after 48 hours.

Parasites implanted into the vocal sacs succumbed usually within 24 hours regardless of whether the vocal sac opening remained open or was plugged. Three of 8 implanted flukes were not present in the vocal sacs at autopsy. Host frogs had been placed in running water and it was not determined whether flukes had

migrated into the mouth cavity and been spit out or swallowed. Flukes recovered at autopsy, 2 to 3 days after implantation, were usually swollen, with marked acetabular hypertrophy, and general disintegration and sloughing of tissues.

Flukes implanted into lungs through lateral incisions or through the glottis failed to survive. Host cells were clustered on the cuticula within 12 to 24 hours even though parasites retained normal activity and histology. Parasites lived longer (up to 24 to 36 hours) implanted through the glottis than through lateral incisions (up to 24 hours); cut blood vessels in the latter may have afforded easier and more copious outflow of phagocytes and erythrocytes. Maximum survival in glottal implants, of approximately 50 hours, occurred when the trachea was plugged and the glottis closed by suture. Control frogs with plugged and sutured tracheae lived, at 18 to 20° C., for at least 1 month without ill effect. Cuticular penetration, histolysis and phagocytosis were similar to those encountered in other implantation sites. Flukes carefully inserted through the glottis into the lung cavity were recovered, in plugged hosts, generally in the bronchi or trachea. Sucker action appeared effective in these, since clusters of ciliated columnar epithelial cells, abraded from the inner lining, appeared regularly in the cellular trash surrounding the parasite.

Gorgoderids placed in the abdominal coelom died within 24–72 hours. Autopsy revealed them adherent to the outer surface of the urinary bladder and kidneys (Fig. 7), also to the dorsal mesentery, the fat bodies, the liver (Fig. 8), and the lungs. Each was firmly encapsulated and partially histolysed by host cells similar to those encountered in the eye. Flukes placed in sealed celloidin bags were also dead and fragmenting within 72 hours. Although these bags were carefully inspected under the binocular dissecting microscope to insure absence of imperfections, at the end of the experiment breaks were observed and corpuscles were present within the bags.

In sharp contrast to the quick death of parasites transplanted to new sites within the host was an unexpected longevity of those placed in saline solutions *in vitro*. Death of flukes immersed in amphibian Ringer's diluted with distilled water to form a series, ranging from 100% to 5% Ringer's, seemed to follow a skewed normal curve. A peak of survival, extending as long as 7 days, occurred in 60%–80% Ringer's. Although the starving flukes became more feeble, spontaneous or induced movements were perceptible during this whole time. Replicate series altered by bubbling nitrogen continuously through the saline solutions gave survivals of more than 13 days in 65% Ringer's. Flukes placed in frog urine were dead in less than 22 hours and those placed in 60% Ringer's in closed systems of reduced oxygen tension, achieved by either alkaline pyrogallol or saline displacement of the air, were usually dead in 36 hours.

In vivo experiments included perfusion of bladders with fluids and gases and implantation of mutilated flukes. Water and Ringer's solutions, up to twice normal salt concentration, dripped into the bladder over a 3–7 day period, were without visible effect on the behavior or appearance of perfused flukes. Air bubbled into the bladder at a rate of approximately 4 cc. a minute altered intravesical conditions so markedly that some flukes were immobilized and were eliminated from the frog within 8½ hours. Within 20 hours parasites still in the bladder were detached and barely alive or were floating dead in the urine. Characteristic dorsal flexure of

the prosoma and hypertrophy of the acetablum were manifest. Although violence is done to flukes by copious *in vivo* aeration, results of a lesser volume of air were not determined. It is possible that reduced amounts of air would not be harmful since *Gorgoderina attenuata* consumes oxygen. Measurements with a capillary respirometer show that about 0.40 cu. mm. of oxygen, from air at normal atmospheric pressure, is consumed by 1 mg. dry weight of organisms (approximately 3 adult flukes) each hour at 21° C. Nitrogen bubbled at a 4 cc. a minute rate into bladders of the same species (*Rana pipiens*) housing the same fluke proved physiologically benign. Two of 17 flukes were mechanically swept from the bladder in the first explosive release of nitrogen which, as in air too, tended to inflate greatly the bladder before bubbling to the outside through the vent. These 2 flukes were in no way different from normal intravesical ones. Spontaneous loss of flukes in forceful urination has previously been reported by Goodchild (1948). Transplanted flukes remaining in the bladder were normal after 50 hours in nitrogen. Seven flukes in which narrow to wide single notches were cut in the ventral sucker before implantation were unable to maintain themselves in the bladder. They were not found there 2 to 3 days after implantation although non-mutilated controls inserted at the same time were found; decomposing worm fragments were regularly seen in the bottom of containers housing experimental frogs.

Discussion

Although successful auto-, homo-, and hetero-transplantation of gorgoderid flukes into bladders was not unexpected, because *Gorgodera amplicava* and *Gorgoderina attenuata* are both somewhat host and organ plastic, their prompt death in non-vesical sites was. Profound differences in oxygen tension, physical substrate, nutritional opportunities, aquosity, and viscosity occur in the experimental habitats; any or all of which may be conditional to early death. It seems hardly possible, however, that alterations in physical conditions can be held solely responsible since *in vitro* longevity, with as great physical modifications, usually exceeded *in vivo* survival. Undoubtedly biotic defenses such as hostile antibodies, and other foreign proteins, invasive phagocytosis, and general tissue immune reactions, aided in the quick death. Mann and Fratta (1953) considered the latter a possible explanation for failure of *Dirofilaria immitis* microfilariae to invade and grow in cats, whereas some adult worms transplanted from dogs survived and larviposited for 52 days.

Successful homo- and hetero-plastic implantations of tissue and organisms have been made into the eye. The conjunctival sac and the anterior chamber are ideal sites for such transplants because translucency or transparency of the outer layer allows periodic or continuous inspection during progressive or regressive changes. Homoplastically implanted embryonic rat tissues including brain (May, 1930), Müllerian duct (Bronski, 1950), ovaries (Goodman, 1934), and prepuberal testes (Turner, 1938a) retained cellular integrity, and the latter two became physiologically active in the anterior chamber. Adult tissues such as rabbit prostate (Heckel and Kretschmer, 1935), anterior lobes of guinea pig and rabbit pituitaries (Haterius, Schweizer, and Charipper, 1935), and rat adrenal glands (Turner, 1938b) also retained normal histology and, in part, normal secretory activity. Histological differentiation of bone, intra-ocularly from marrow of a mouse's

femur, was reported by Pfeiffer (1948). Pathological tissue has been successfully maintained in the anterior chamber (Nakayama, 1927, and others). Successful heteroplastic implantation of thyroid (Bisgard, 1938), of *Treponema cuniculi* (Van Canneyt, 1929), and avian tumors (Shrigley, Greene, and Duran-Reynals, 1947), were made into anterior chambers. Fawcett, Wislocki and Waldo (1947) found a phagocytosis, strikingly like that initiated by implanted bladder flukes, induced by homoplastically implanted mouse zygotes. Although the former did not survive beyond 22 hours, zygotes developed for 15 days before being destroyed.

Concomitant with phagocytic diapedesis and erythrocytic extravasation was engorgement and proliferation of blood vessels in the iris and cornea. Histological degradation of iris subjacent to the parasite was also apparent. These responses, which are not elicited by implanted endometrium, mammary gland, and thymus (Fawcett, Wislocki, and Waldo, 1947), may be induced by specific mechanical or biological irritations from bladder flukes.

Incompatibility between host and flukes implanted in the coelom and dorsal lymph space also resulted in almost immediate death of parasites. These habitats are like the anterior chamber in being perfused with lymph-like fluids and cells originating from blood. In both, as in the eye, parasites became immeshed in a stroma of phagocytes and fibers. Survival difficulties in the coelom have interesting phylogenetic speculative value. *Gorgodera* and *Gorgoderina* belong to the subfamily GORGODERINAE, which with the subfamily ANAPORRHUTINAE, comprise the family GORGODERIDAE. The latter subfamily consists chiefly of genera residing in the coelom of CHONDRICHTHYES. Although no life cycles or larvae are as yet known for anaporrhutines it is possible that metacercarial excystment in the intestine is followed by a posterior migration to the cloaca and coelomic invasion by the abdominal pores. Skates and rays, frequent hosts of anaporrhutines, are mollusc feeders and are descendants of remote fresh water ancestors. If anaporrhutines and gorgoderines are phylogenetically related, their divergence from a common freshwater origin must have occurred in late Ordovician or early Silurian with the emergence of CHONDRICHTHYES. Moreover if anaporrhutines, being in phylogenetically older hosts, are ancestral then gorgoderines have lost an original ability to reside in the coelom.

Bladder flukes seem to be euryanoxybiotic aerofermenters which, like *Ascaris* (Laser, 1944), are injured by high oxygen tensions. Elimination, often by spontaneous evacuation, and death occurred when flukes were placed in the vocal sacs and lungs. In the latter, longevity could be lengthened slightly by plugging the trachea to prevent entrance of air. Air bubbled into the urinary bladder was deleterious and caused evacuation and death as early as 8½ hours; by 20–30 hours all flukes were dead. Nitrogen introduced, for 50 hours, in the same manner and at the same rate did not affect longevity or behavior. Unfortunately little is known concerning *in vitro* survivals, aerobically or anaerobically, for DIGENEA (cf. von Brand, 1946), and less is known about survival in experimentally altered *in vivo* habitats. Gorgoderids thrive in the lumina of organs having little dissolved free oxygen, and yet they will consume it when available to them. Their rate of consumption, 0.40 cu. mm./1 mg. dry wt./hour is similar to that for *Ascaris lumbricoides* (0.33–0.82 cu. mm.), *Diphyllobothrium latum* plerocercoids (0.34

cu. mm.), *Eustrongylides ignotus* larvae (0.56 cu. mm.), as summarized by von Brand (1952).

Flukes were tolerant of some other changes in their natural intravesical environment. Physiological salt solutions, up to twice normal salt concentration, and distilled water were without harmful effects.

Experiments on survival of flukes in *in vitro* saline solutions are preliminary only. Normal urine proved lethal in less than 24 hours, whereas 60%–80% amphibian Ringer's solution permitted survivals up to 7 days. The same solution devoid of air, by displacement with bubbling nitrogen, supported life for 13 days. In each of these series food and substrate may be more important survival factors than saline concentration. The languidness, *in vivo* and *in vitro*, of flukes denied suitable surfaces for attachment suggests that their permanent *in vitro* maintenance will depend, in part, on satisfying their needs for proper substrates. Smyth (1953) at last succeeded in his attempts to obtain fertile eggs from cultured *Schistocephalus solidus* by supplying a sterile artificial intestine made from cellulose tubing whose walls could be compressed to assist impregnation and through which dialysis of wastes could be achieved. For bladder flukes thin latex, thin celloidin sheets, vaseline, agar-agar, cellulose sponge, coverglasses, and thin cellulose tubing have already been tried without success.

An enigma of *in vivo* results has been the consistent failure of flukes to achieve any normality of behavior for longer than a day or two. And yet gorgoderid penetration and maturation will occur in the mesonephros (Odlaug, 1937, Goodchild, 1950) an organ fully as vascular as any in the body. In this extravesical site penetration occurs by the juvenile and although leucocytic mobilization begins and contact is made, cuticular breakthrough is not achieved and the parasite succeeds in establishing itself. Host encapsulation walls off the parasite but does not kill it. Juveniles may differ from adults in important biological ways other than size, although the latter alone may be effective in inducing accelerated phagocytosis. Fawcett, Wislocki, and Waldo (1947), suggested that the host responses are correlated with bulk of foreign implant; survival expectancy in the eye was less with many mouse zygotes than with one. Although operations and implants were performed oligoseptically, bacteria introduced with the worm may contribute to the prompt, efficient phagocytosis and early demise of the parasite. Bacterial multiplication in organically rich urine may also result in pH and other changes which prove rapidly lethal *in vitro*.

Summary

Adult bladder flukes, *Gorgodera amplicava* from *Rana catesbeiana* and *Gorgoderina attenuata* from *R. pipiens* were successfully auto-, homo-, and hetero-transplanted into bladders of these anurans and *R. sylvatica*. Forty flukes were normal in behavior and appearance 21 days post-implantation.

Homo- and hetero-transplantations to conjunctival sacs and anterior chambers of frog eyes resulted in prompt death of parasites. Within 4 hours phagocytes were mobilized and were collecting around and on the parasite. In whole mounts and *in toto* stained sections the course of diapedesis and extravasation from iridal and corneal vessels of monocytes, basophiles, lymphocytes, and approximately 1% of eosinophiles was followed. All flukes were dead and invaded by leucocytes within

24 hours. Continuing histolysis and infiltration reduced the parasite, after 96 hours, to an amorphous remnant. The anterior chamber was modified as a result of implantation by engorgement and tortuosity of iridal and corneal blood vessels, by copious extravasation of leucocytes and erythrocytes, and by degradation of iridal chromatophores.

Similar phagocytic responses and early deaths of parasites were recorded for implants into the vocal sacs, dorsal lymph space and coelom. In these habitats death might be delayed for 24 hours, but was certain by 48 hours. Aggregations of phagocytes, preceding cuticular penetration, were most numerous, often 10–20 or more cells thick, on the post-acetabular body.

Lung implants were also killed and phagocytes were clustered about the cuticula by 24 hours, although in a few instances flukes might appear normal for 48 hours. Implants into lungs through lateral incisions were less successful than those made through the glottis. Slightly greater longevity, more normal behavior, and greater percentages of recovery were obtained from lung segments tied off from the trachea, or better still, from lungs with tracheae plugged to prevent entrance of air.

Starving flukes could remain alive in 60% to 80% Ringer's solution for 7 days. When nitrogen was continuously bubbled into the same saline solution they lived for 13 days. In the host, normal to twice concentrated Ringer's solution perfused into the bladder was without effect; nitrogen gas bubbled into the bladder also was without visible effect on behavior or survival. Oxygen, even though used (0.40 cu. mm./1 mg. dry wt./hour), caused death or evacuation from bladders into which air was bubbled. The latter effects also resulted from minor or major mutilation of the ventral sucker.

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EXPLANATION OF PHOTOMICROGRAPHS

Abbreviations: AC, anterior chamber; BV, engorged blood vessel; C, cornea; E, erythrocytes; FS, fibrous sheath; IR, iris; L, crystalline lens; LI, liver; M, myxomatous matrix; MI, miracidia; P, host phagocytes; S, sucker.

PLATE I

Figs. 1 through 6 are of *Gorgoderina attenuata* homotransplanted into the eye of *Rana pipiens*.

FIG. 1. Frontal section, 4 hours post-implantation. Host phagocytes are in small clusters on the cuticula and in strands oriented toward the fluke.

FIG. 2. Cross section, 16 hours post-implantation. Engorged iridal blood vessels, breakdown of iridal chromatophores, and numerous host phagocytes in a myxomatous matrix embedding the parasite are apparent. Normal fluke histology has largely disappeared.

FIG. 3. Frontal section, 36 hours post-implantation. Conspicuous is loss of integrity of cuticular and internal organs. Other salient features include cellular adhesion to a slightly degraded iris also marked with vascular engorgement on the outer margin, and the fibrous and cellular matrix.

FIG. 4. Sagittal section, 36 hours post-implantation. Note layer of phagocytes on basement membrane of ventral sucker, degradation of iridal chromatophores, phagocytosis of fluke, and diapedesis of host cells. Adhesion, hyperplasia, and slight vascular engorgement are visible in the cornea.

PLATE II

FIG. 5. Sagittal section, 48 hours post-implantation. Host cells permeate the whole parasite including musculature and lumina of the suckers. Reproductive organs are marked by aggregations of host cells. Extravasated erythrocytes occur in a well-defined layer in the fibrous matrix.

FIG. 6. Cross sectional area, 96 hours post-implantation. Outer and inner fibrous matrices are dense and confluent. The musculature of the sucker is being invaded by phagocytes. Innumerable erythrocytes surround the parasitic remnant.

FIG. 7. Cross section of *Gorgoderina attenuata*, 72 hours post-implantation into the coelom of a female *Rana pipiens*. The parasite is encapsulated in a fibrous and cellular sheath adherent to the urogenital system.

FIG. 8. Section of *Gorgoderina attenuata*, 72 hours post-implantation into the coelom of a female *Rana pipiens*. The parasite is encapsulated in a fibrous and cellular sheath adherent to the liver.

PLATE I

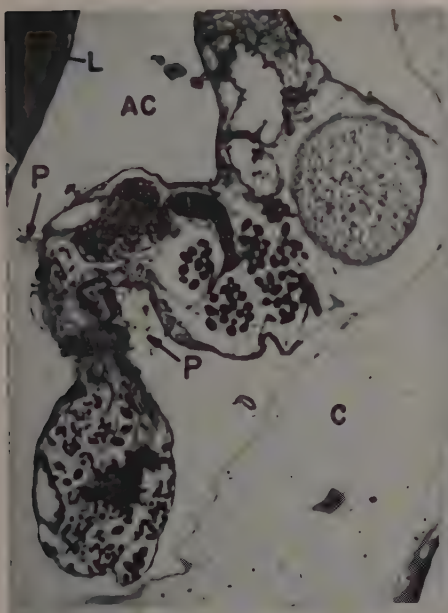

FIG. 1

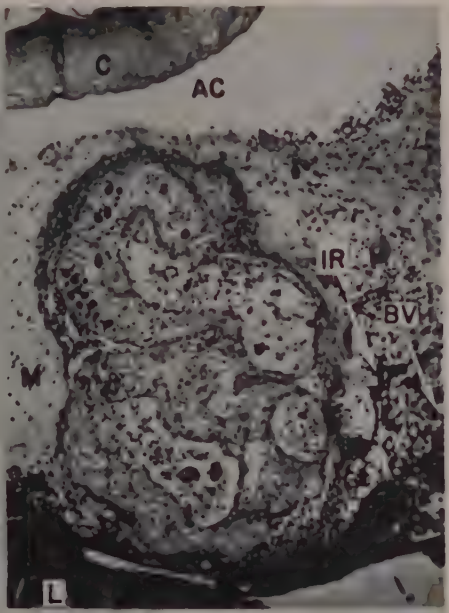

FIG. 2

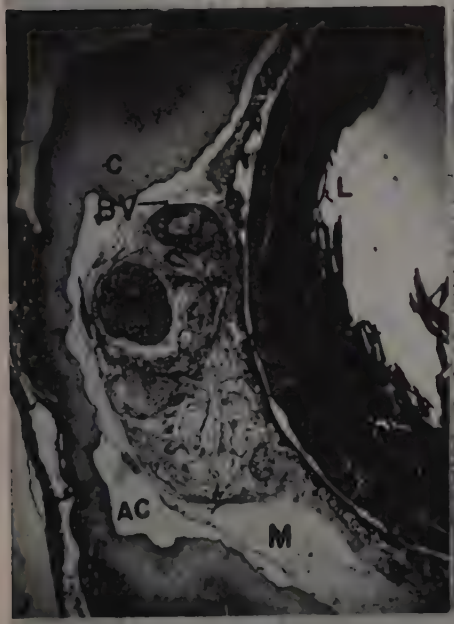

FIG. 3

FIG. 4

PLATE II

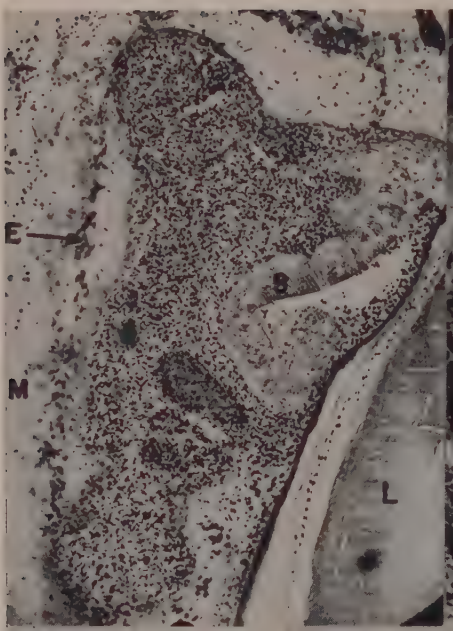

FIG. 5

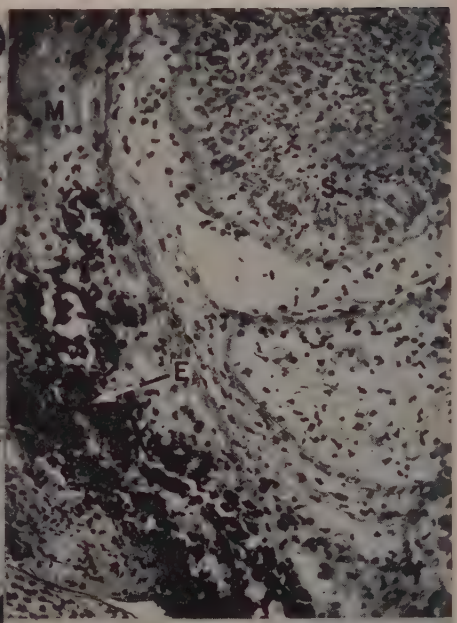

FIG. 6

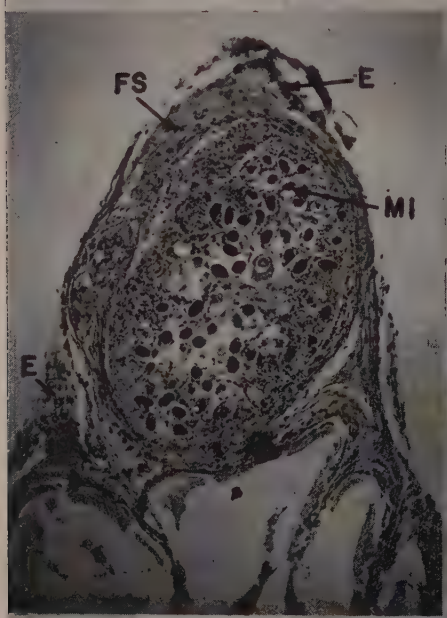

FIG. 7

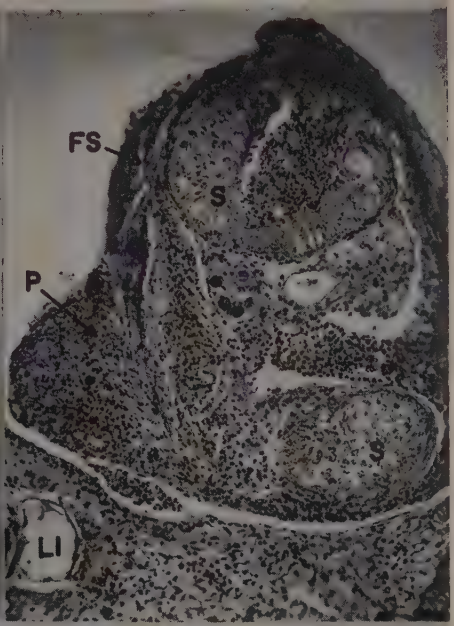

FIG. 8

REINFECTION AND SEASONAL FLUCTUATIONS OF *ASCARIS LUMBRICOIDES* AMONG A GROUP OF CHILDREN IN AN AREA WHERE NIGHT SOIL IS USED

CHIA-TUNG PAN,[1] L. S. RITCHIE[2] AND G. W. HUNTER, III[3]

The 406th Medical General Laboratory,
APO 500, c/o Postmaster,
San Francisco, California

INTRODUCTION

The benefits of single treatments for *Ascaris lumbricoides* have been shown to be of short duration in areas where infections are perpetuated by gross pollution of dooryard and environs (Cort, Schapiro and Stoll, 1929; Otto, 1930; Otto and Cort, 1934; and Headlee, 1936). In countries where vegetables are fertilized with night soil but where promiscuous defecation is not practiced, the effectiveness of single treatments on the control of *Ascaris* infection has not been clearly established. As a corollary of an evaluation of vermifuges currently used against *Ascaris* in Japan, observations were made to determine how quickly the pre-treatment level of infection with this parasite would be re-established among a group of teen-age children who had ceased to discharge eggs following treatment. The study also afforded observations on seasonal fluctuations in incidence and numbers of eggs discharged, which appear to be correlated with certain epidemiologic conditions (Pan, Ritchie and Hunter, 1953).

Repeated administration of anthelmintics to large groups has not been practiced in Japan due at least in part to inherent dangers of known effective drugs. Recently several Japanese workers have investigated the possible benefits that might result from repeated doses. Nagano (personal communication), dealing with an isolated population of about 300 on the island of Hatsushima, reports a considerable number of persons still lightly infected after a two-year period of monthly examinations and coincident treatment of those discharging eggs. Similarly, Hoshi and Morizaki (1952) and Yamada (1952), dealing with groups within larger populations, gradually lowered prevalence and density by repeated treatments; but complete elimination was not attained.

METHODS

A group of 282 teen-age children (12–15 years of age) was selected in Yamanashi Prefecture, Japan, from among a larger number (405) which had been treated with hexylresorcinol (a dosage of 1 gram, followed by a sodium sulfate purge 3 hours later). Each child selected was negative for *Ascaris* eggs on 4 consecutive stool examinations made during February 1950, 2–4 weeks after medication. Following treatment, monthly examinations were made for 14 months (February 1950 to March 1951). The prevalence of *Ascaris* before treatment was 78% and the parasite-density index, 134 (Tigertt, Hunter and Ritchie, 1952). Although the

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[1] 406th Med. Gen. Lab. and Harvard School of Public Health, Boston, Mass.

[2] Dept. of the Army Civilian; Professional Adviser to the Department.

[3] Colonel, MSC, U. S. Army; formerly Chief of Dept.; at present Chief, Section of Parasitology-Entomology, Fourth Army Area Medical Lab., Fort Sam Houston, Texas and Professor of Parasitolgy, Affiliated Units of the Graduate School, Baylor University.

group was not a random sample of the population, it has been observed that incidence and density are relatively uniform for various age groups in Yamanashi Prefecture (unpublished data); consequently it is believed that the results are actually indicative of the rapidity of reinfection for the general population in this area.

A single stool was requested each month, and of the 282 cooperating persons, the following numbers submitted a specimen for the 14 consecutive months: 282, 282, 266, 261, 205, 219, 203, 211, 203, 194, 184, 155, 150 and 40. The AMS III technic (Hunter, Hodges, Jahnes, Diamond and Ingalls, 1948) and the same technicians were used throughout the study, providing a uniform base-line for comparison. In addition, fluctuations in the egg-count were determined by means of the parasite-density index of Tigertt, Hunter and Ritchie (1952).

RESULTS

By the end of the second month following treatment (March), 5% of the original individuals had begun to discharge eggs (Fig. 1.) This percentage was more

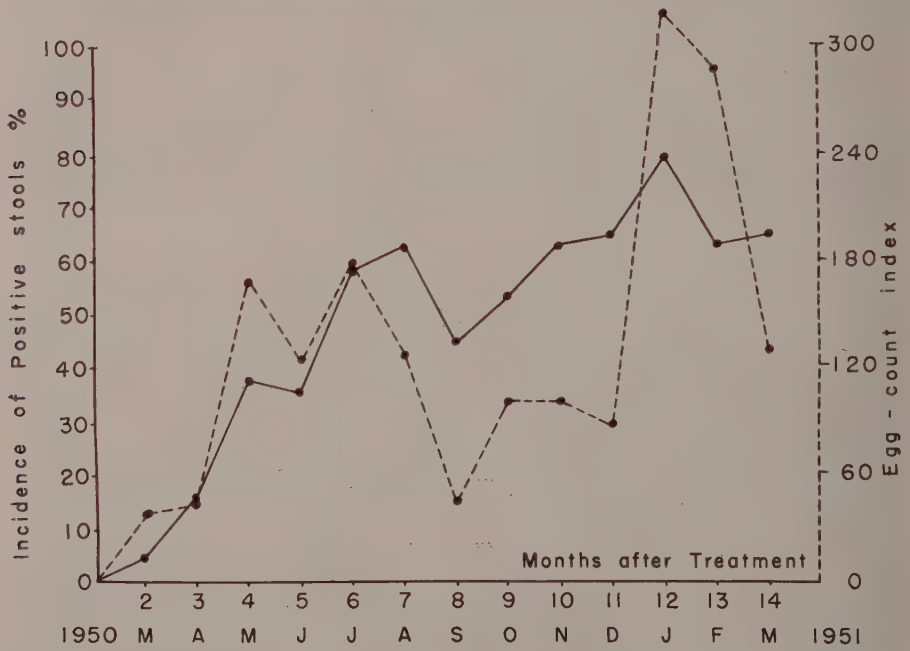

FIG. 1. Reinfection by *A. lumbricoides* of Japanese school children in Yamanashi Prefecture, Japan. The solid line represents incidence; the broken line represents the egg count, or parasite-density index.

than tripled in April (17%), and this more than doubled in May (38%). There was no significant change during June. The greatest rise in incidence came in July with an increase to 59%, followed by a limited increase for August (64%). In September there was a sharp decline in the number of individuals from whom eggs were recovered (45%). From this point on, there was a steady increase through January, 1951, when the pre-treatment infection level (78%) was reached. Changes in the density index of egg production also occurred which tended to parallel fluc-

tuations of incidence. There were increases in the number of eggs discharged during the spring and summer, followed by a marked decrease in September. Subsequently, through December, the parasite-density index lagged behind the increase in occurrence of positive stools; however, by January 1951, figures for both reached maximum levels, at which time the occurrence of positive stools equalled the pre-treatment level, while the parasite-density index was more than double the original (Fig. 1).

DISCUSSION

The parasite density index is a numerical value of the average number of eggs per gram of stool based upon actual egg counts. This has been correlated with the Stoll egg dilution count and gives a fairly accurate indication of the number of worms present (Hunter, Ritchie, Nagano and Ishii, 1951; Tigertt, Hunter and Ritchie, 1952; Hunter, Ritchie, Kaufman, *et al.*, 1952). The decline in the parasite-density index as well as the occurrence of stools positive for *Ascaris* in the early fall was sharply defined, clearly showing a peak in midsummer (July and August) and a maximum level in midwinter (January and February). Whether the infection level fluctuates between February and July or remains at a maximum level was not clearly evident from the data. However, the observations suggest that at least during the fall months, worms are expelled faster than they are acquired by reinfection. Among the several means of transmission postulated for *Ascaris* in Japan, the consumption of raw and pickled vegetables is one of the most probable explanations for these findings. Seasonal availability of vegetables most apt to account for transmission, combined with climatic conditions and practices in the use of night soil, may well account for the seasonal fluctuations in the *Ascaris* infection.

The pre-patent period of *A. lumbricoides* in man is about 2 months (Ransom, 1920; Koino, 1922; and Takata, 1951). The presence of an abundance of embryonated eggs in the environment must, therefore, antedate peaks of infection by at least 2 months, which according to our findings would be approximately May and November. According to Ochi (1932) eggs discharged from the body in winter (November to March) are retarded in development, and those which survive do not reach the infectious stage until late spring. Applications of night soil made during the late fall, winter and spring might tend to account for an accumulation of embryonated eggs in the soil and on the vegetables by late spring. The first crops of the year include cabbage, spinach, green onions, radishes and turnips, which are in large part consumed raw and/or pickled. As much as 80% of such vegetables as sampled by Yamaguchi (1925) and Sugita (1926) were found to be contaminated by viable *Ascaris* eggs. More recently, Fueki (1952), examining 2250 samples of 20 kinds of vegetables, not only noted that infectious *Ascaris* eggs occurred commonly on leafy and root vegetables, but that their numbers increased during the spring months. Thus, a combination of food habits, farming practices and climatic conditions exist in Japan which could account for excessive exposure in the late spring and a peak of infection in July. The above mentioned conditions are universal throughout Japan except in extreme northern parts.

The apparent decline of ascariasis in the fall may well be linked with the observation of Sugita (1926) and Fueki (1952) that the highly contaminated leafy and root vegetables of spring are replaced in the summer diet by vegetables such as

tomatoes, eggplant, cucumbers, peas, beans, corn, etc., which according to the findings of Fueki are rarely contaminated. Furthermore, Ochi, while noting a rapid rate of development in July and August also observed a reduction in the number of viable *Ascaris* eggs during the same period. Otto (1929) also found that eggs exposed to sunlight during the summer became desiccated rapidly and died, depending partly upon the type of soil and humidity. Takasaki (1935) showed that exposures to direct sunlight (28–42°C.) for 7 or more hours will render the eggs noninfectious. Fueki's data also suggested the reduction in midsummer (August) of embryonated as well as viable eggs.

A maximum level of infection in the winter months (January and February) can likewise be correlated with the influences of food habits, farming practices and climate. With the coming of fall, there is the reappearance of leafy and root vegetables. Although the egg density of the soil may decrease under the influence of summer temperature, some of the eggs in night soil applied to the fall vegetables in late August and September probably become infective before cold weather. This is confirmed by Fueki (1952) who recovered infective eggs from fall vegetables (to be eaten either raw or pickled) in numbers equalling the spring peak (*vide supra*). Ogata (1922), and Morikawa and Fukuda (1937) have reported the presence of embryonated eggs in pickles, which are a very important part of the daily diet throughout the year, but especially so in the fall and continuing throughout the winter and spring. The increase in incidence and egg output which we noted in January and February is consistent with the greater contamination of fall vegetables, and the required pre-patent period.

By this correlation of seasonal fluctuations in *Ascaris* with food habits and farming practices, it is not intended to deny that other modes of transmission are involved; however, their contribution to such a fluctuation appears less important judging from the large quantity of raw and/or pickled vegetables consumed daily. The possibility of *Ascaris* eggs being dust-borne should especially be mentioned as highest wind velocities prevail during the dry months of April and May, which antedate the summer peak of *Ascaris* burden by two months, which is the length of the pre-patent period. This may be discounted to some extent by the fact that winds and dryness do not correspondingly antedate the winter infection peak. The relative importance of the dust-borne theory has not been established, but Nishimura (1952) and Fueki (1952) have recovered considerable numbers of viable eggs from dust collected in schools and homes. The latter reported that the total number of eggs in 100 one-gram dust samples equalled 44% of the eggs recovered from comparable soil samples. From the 100 samples, 121 eggs were recovered of which 48% were embryonated. According to these findings about 1 egg would be taken into the nose or mouth with each 2 grams of inhaled or ingested dust, and not all of these would necessarily be swallowed and mature to adult worms. Finger transmission appears likely as infective eggs have been recovered from fingers and dirt under the nails by a number of Japanese investigators. Adults working in the soil might expose themselves to infection as well as children playing in the dirt. Although isolated stools are sometimes spotted around dooryards, promiscous defecation is not practiced. All homes are equipped with some type of toilet which is consistent with the need for the excreta as fertilizer. Consequently gross soil pollution is infrequent, except possibly at the back of the toilet where night soil is dipped.

The seasonal fluctuations noted in this study are in accord with the conclusion of Cort, Schapiro, Riley and Stoll (1929) that *A. lumbricoides* is very easily voided spontaneously and that it is necessary to have a constant source of infection to maintain a high incidence. The marked decrease in prevalence and intensity of *Ascaris* infecton in the fall, preceded and followed by maximum levels in the summer and and winter, may indicate the balance between reinfection and spontaneous voiding of this parasite. As this group was treated at the beginning of the present study and all were essentially free of ovipositing worms, the decline in the prevalence in September suggests that the average life span may be considerably less than one year. Otto (1930) stated that spontaneous passage of *Ascaris* is not uncommon, and that there is almost a complete turnover of the worm burden each year.

SUMMARY

Reinfection with *A. lumbricoides,* following treatment, has been investigated in Japan where night soil is extensively used in vegetable gardens. Monthly examinations were made on a group of teen-age children over a period of 14 months. Within 2 months after treatment reinfections began to appear, approaching the original incidence and intensity of infection after 1 year. A sharp decline in the level of both incidence and the parasite-density index of *Ascaris* occurred in September. Maximum levels of infection existed during the winter and summer months, between which a record decline in the spring may have occurred. Possible explanations for these seasonal fluctuations are based upon the combined effect of seasonal variations in diet and time of applying night soil. The findings confirm a relatively short life span for *Ascaris lumbricoides.*

ACKNOWLEDGMENTS

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THE LIFE CYCLES OF NEMATODES IN THE FAMILY ASCARIDIDAE BLANCHARD 1896[1]

J. F. A. Sprent

University of Queensland Veterinary School, Yeerongpilly, Brisbane, Queensland, Australia.

The use of intermediate hosts among the Ascarididae.

Leuckart (1866) suggested that certain members of the family Ascarididae utilize intermediate hosts; the present paper summarizes the evidence which has subsequently accumulated and discusses this aspect of the life history in relation to the evolution of the group.

(a) Fish.

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[1] This paper was read in part before the American Society of Parasitologists at Madison, Wisconsin in September, 1953. The writer wishes to express his thanks to Dr. J. D. Tiner for presenting the paper.

Wülker (1929) observed that various marine fish in Norwegian waters, especially herring, harbored in their tissues larval ascaridids resembling *Acanthocheilus quadridentatus* from sharks and rays, and that various flatfish harbored larvae resembling *Contracaecum* spp. from the large gadid fishes, seals and marine birds. He referred to the discovery by Apstein (1911) of nematode larvae in the body cavity of the plankton copepod, *Calanus finmarchicus,* and having re-examined Apstein's material, was convinced that they were *Contracaecum* spp. He considered that there might be three hosts in the life history, i.e. (1) copepod, (2) plankton-eating fish, (3) piscivorous fish, birds or mammals. Larval ascaridids were observed in *Sagitta* by Pierantoni (1914) in the Bay of Naples and by Lebour (1917) in *Sagitta bipunctata* in Plymouth Sound. Freitas and Lent (1946) found the larvae in the body cavity of *Diaptomus.* Layman (1935) observed that larval stages of *Anisakis, Porrocaecum* and *Contracaecum* occurred in various tissues of fish in the Arctic Sea. Kahl (1936) considered that the whiting, *Gadus merlangus,* was the usual intermediate host of *Contracaecum clavatum,* a common parasite of the cod, *Gadus morrhua,* in the North Sea.

Markowski (1937) working with *Contracaecum aduncum* from *Zoarces viviparus* from the Baltic Sea, demonstrated that the embryonated eggs were devoured by the plankton shrimps, *Eurytemora affinis* and *Acartia bifilosa,* and that further development occurred in the body cavity. Janiszewska (1937) found the third stage larvae of this parasite encysted in the intestinal wall, liver and mesenteries of the flounder (*Pleuronectes flesus*); free third and fourth stage larvae were frequently found in their intestines, but no mature adults. She concluded that development from hatching to the encysted third stage larva took place in an intermediate host which might be an invertebrate or a fish. Thomas (1937c, 1940) found that dragon fly nymphs (*Tetragoneuria* spp.) and small fishes (guppies) swallow the embryonated eggs of *Raphidascaris canadensis* from the pike (*Esox lucius*), and that development proceeded to the infective stage in the liver of guppies. He concluded that infection of the final host occurred through eating these intermediate hosts. Layman (cited by Mozgovoi, 1953) has confirmed the conclusion by Leuckart (1866) that the larvae of *Raphidascaris acus* use small cyprinid fishes as intermediate hosts.

(b) Reptiles.

Ortlepp (1922) demonstrated that larvae from the eggs of *Polydelphis attenuata* from *Python spilotes* migrated through the liver and lungs of mice. Walton (1937) observed larval nematodes encysted on the mesenteries and body muscles of various amphibia. He fed infected amphibia to snakes (*Natrix rhombifera* and *Natrix sipidon sipidon* [sic]) and obtained infection with *Ophidascaris labiatopapillosa;* he infected an alligator (*Alligator mississipiensis*) with *Multicaecum tenuicolle* by the same method. The present writer (unpublished) infected mice with embryonated eggs of *Ophidascaris filaria* from *Python spilotes,* and observed considerable growth of the larvae in the liver; the larvae were also found encysted in the various parts of the general musculature, especially the forelimbs. The mice were fed to an uninfected *Python spilotes* which soon afterwards went into hibernation. Four months later, at the commencement of the hot weather, it died and it was found to contain enormous numbers of larvae in various stages of development from mature

specimens in the intestine to third stage larvae situated in various parts of the body. Many of the larvae were encysted in the lungs, muscles and intestinal wall.

(c) Birds.

R. Leuckart (1876, p. 98) suggested that larval ascaridids found encysted on the abdominal viscera of moles were immature stages of *Porrocaecum depressum,* a parasite of hawks and owls. These larvae, first reported by Goeze (1782, p. 130, Plate VIII, figs. 7 and 8), were named *Ascaris incisa* by Rudolphi (1802, p. 20), who also described (p. 29) *Ascaris pusilla* from the peritoneal cavity of the hedgehog. F. Leuckart (1842, p. 42) and Creplin (1844, p. 132) found encysted ascaridid larvae on the abdominal viscera of shrews (*Sorex tetragonurus*) ; the former named this parasite *Ascaris acanthura.* Schwartz (1925) described two larval nematodes from the subcutaneous tissues of insectivores; the larva from *Scalopus aquaticus* (a mole) was named *Porrocaecum americanum* and the larva from *Blarina brevicauda* (a shrew) was named *P. encapsulatum;* Schwartz concluded that the adult parasites occur in hawks and owls. Chandler and Melvin (1951) found larvae, resembling *P. encapsulatum* Schwartz, in the subcutaneous tissues and in the abdominal cavity of *Blarina brevicauda* and *Parascalops breweri* (a mole). They found larvae resembling *P. americanum* Schwartz on the abdominal viscera of *Blarina brevicauda, Sorex* (*fumaris?*) and *Parascalops breweri.* Those authors stated that the larva of *P. americanum* Schwartz may be identical with *A. incisa* Rudolphi. Encysted larvae identified as *Porrocaecum* spp. have been found by Baylis (1928) in the axilla of Nigerian shrews (*Crocidura* sp.), by Solonitzyn (1933) on the serous surface of the stomach of Russian desmans (*Desmana moschata*), by Pologentsev (1935) in the skin and abdominal viscera of the shrew-mouse (*Sorex araneus*), and by Rector (1934) in the salivary glands of moles.

The experiments of Dubinin (cited by Mozgovoi, 1953) have indicated that herons (*Ardea* spp.) become infected with *Contracaecum microcephalum* and *Porrocaecum reticulatum* by feeding on fish harboring encysted larvae in their tissues. According to Mozgovoi (1953) ducks become infected with *Porrocaecum crassum* by swallowing earthworms which harbor developing larvae in their blood vessels.

Thomas (1937a, b and 1940) observed that the smaller specimens of *Contracaecum spiculigerum* harbored by cormorants (*Phalacrocorax auritus auritus*) were identical with larvae encysted on the mesenteries of the gizzard shad (*Dorosoma cepedianum.*) He demonstrated that the eggs of this parasite hatched spontaneously in water and that the larvae were ingested by tadpoles and by guppies (*Labistes reticulatus*), in whose mesenteric tissues the larvae became encysted. Fledgling cormorants were infected by feeding on infected guppies.

(d) Mammals.

According to Baylis (1916), Stiles and Hassall (1899) observed that larval nematodes, encysted in the tissues of marine fishes resembled *Porrocaecum decipiens* of seals and walruses, indicating that infection of the final host occurs through eating fish harboring the encysted larvae. Soodarikov and Ryjikov's work (cited by Mozgovoi, 1953) indicated that *Contracaecum osculatum* from *Phoca sibirica* uses the pelagic crustacean, *Macrohectopus branickii,* and the yellow-winged bullfish (?) as intermediate hosts.

The utilization of intermediate hosts among the ascaridids of terrestrial mammals was not demonstrated until recently, although such had been tentatively suggested

by Leuckart (1876) for *Toxocara mystax,* and by Fülleborn (1921b) and Hoeppli, Feng and Li (1949) as a possible method of infection for *T. canis.* Tiner (1949), working with *Ascaris columnaris,* discovered that the larvae become encysted in the tissues of rodents and that infection of the skunk resulted from ingestion of infected mice. Petrov [cited by Mozgovoi, (1953)] infected foxes with *Toxocara canis* by feeding them white rats harboring encapsulated larvae in their tissues. Sprent (1952, 1953b) demonstrated that two distinct kinds of migration occur in mice experimentally infected with various ascaridid larvae: the larvae of *A. lumbricoides, A. suum* and *Parascaris equorum* followed a predominantly tracheal migration, the larvae being eliminated from the mouse via the intestines, while the larvae of *A. columnaris, A. devosi, Toxocara canis, Toxocara mystax, Toxascaris leonina* and *Toxascaris transfuga* followed a predominantly somatic migration being distributed to various tissues of the body, where they became encapsulated and remained in a viable condition. The complete development of *A. devosi,* was followed through the white mouse and the ferret (Sprent, 1953a); the larvae of *Toxocara mystax, Toxocara canis* and *Toxascaris leonina* were shown to grow in the intestine of the final host after ingestion of larvae encysted in the tissues of mice (Sprent, 1953c).

Significance of intermediate hosts in the evolutionary history of the Ascarididae.

Fülleborn (1920) suggested that the migration of ascaris larvae through the liver and lungs occurred because this parasite was descended from ancestors whose larvae penetrated the skin. Later (1927), he advanced the alternative view that the larvae migrated through the tissues because they were descended from ancestors which utilized an intermediate host; this view was supported by Cameron (1931). Vinnitsky (1945) criticized the latter viewpoint and, favoring Fülleborn's first suggestion, stated: "The evolution of Ascaridata did not proceed by way of 'omission' of the one of the two hosts, but, on the contrary, by way of 'including' into their life cycle intermediate facultative hosts." Chitwood (1950) stated: "The intermediate host is known to occur only in one subfamily of the Ascaridoidea, the Anisakinae", and, referring to Tiner's work on *A. columnaris:* "It would appear that here we have the first step in the evolution of an indirect life cycle in the Ascaridoidea." It is evident that he did not regard the utilization of intermediate hosts as a primitive character in the Family Ascarididae; moreover, he suggested that entrance of the Anisakinae into fish might have occurred when they came up rivers to feed and denied any possibility of marine origin for the ascaridids.

Dougherty (1951) discussed the evolution of the suborder Ascaridina, and assumed that they began as parasites of the Arthropoda. He suggested that the Oxyuroidea became secondarily adapted to vertebrates from ancestors in arthropods, while the Ascaridoidea arose from a primitive oxyuroid stock within vertebrates, probably after the evolution of the Amphibia. While this appears to be logical reasoning with regard to the Oxyuroidea, there appears to be little evidence to support his suggestion regarding the Ascaridoidea. The main point at issue is the question: Are the members of the family Ascarididae primarily terrestrial, with secondary radiation among marine animals, or are they descended from marine ancestors with secondary radiation amongst terrestrial animals? It appears that

both Chitwood and Dougherty would answer the first part of the question in the affirmative and deny the possibility of the second part.

The present writer, while he does not intend to question the original affinities of the Ascaridoidea with the primarily terrestrial Class Phasmidea, presents the view that the family Ascarididae evolved, not through the terrestrial arthropods and vertebrates, but through the marine arthropods and vertebrates. The evidence in support of this view is as follows: (1) the finding of the adult form of *Toxocara pearsei* by Chitwood (1935) in the intestine of the marine snapping shrimp, *Synalpheus brooksi;* (2) the utilization of marine planktonic crustacea as intermediate hosts by certain ascaridids of fish; (3) the frequent utilization of fish as intermediate hosts by the ascaridids of marine birds and mammals; (4) the broad radiation of the anisakines among marine vertebrates; (5) the observation by Layman (1935) that the development of the esophagus in several genera of the marine anisakines passes through an 'anisakis-stage', indicating their origin from a primitive form resembling the marine genus *Anisakis.*

Such evidence indicates the probability that the family originated as parasites of marine arthropods and have expanded through the utilization of plankton and small fish as intermediate hosts to the piscivorous marine fish, birds and mammals. The spread to terrestrial and freshwater animals possibly occurred through such semiaquatic hosts as the seal, otter, gull and cormorant. All of these harbor ascaridid parasites and may, by depositing the eggs in their feces during their inland excursions, have infected coprophagous animals, such as mice, with consequent lodgement of larvae within their tissues. This method of spread would account not only for the broad radiation of the ascaridids among the carnivorous terrestrial vertebrates which prey upon animals likely to harbor encysted larvae, but also for the characteristic hatching and migration of ascaris larvae in mice.

Modifications of the migratory behavior of the larvae in non-carnivorous hosts.

In extending their host-range to non-carnivorous hosts, different methods of infection have been adopted and the change has involved a variation in the migratory pathway taken by the larvae after leaving the intestine.

a. tracheal migration.

The first of these modifications appears to be a change from the somatic to the tracheal type of migration. It seems likely that the somatic migration is the most primitive one, enabling the larvae to reach the final host through being eaten with the prey, whereas the tracheal migration is probably an adaptation to a non-carnivorous host, allowing the eggs to hatch and the larvae to mature in the same host. The tracheal type of migration has been demonstrated in the final host for *A. suum,* by Stewart (1918); *A. lumbricoides,* by Koino (1922), *A. tarbagan* (in *Marmota siberica*) by Dubinin [cited by Mozgovoi (1953)], and *P. equorum,* by Hadwen (1925). The tracheal migration furthermore introduced the possibility of transport hosts into the life history; Stewart (1918) first suggested that after tracheal migration in rodents, the larvae of *A. suum* may pass out in the feces in a stage infective for pigs. He failed to infect pigs with larvae from the lungs and intestines of mice, but Buckley (1931) succeeded in infecting pigs with larvae from the lungs of a pig. Yoshida (1919) successfully infected himself by swallowing larvae of

A. lumbricoides from guinea pig lungs, but the writer failed to infect himself with larvae of this species from the intestine of mice (Sprent, 1953c).

b. migration to fetus.

A second adaptive modification to a non-predatory host is prenatal infection. First demonstrated by Fülleborn (1921a), this appears to be a frequent method of infection for *T. canis* (Petrov, 1941, Nifontov, 1949) and has been shown to occur also with *Neoascaris vitulorum* in calves by Davtian [cited by Mozgovoi (1953)]. Observations have so far indicated (unpublished) that prenatal infection of kittens with *T. mystax* does not occur; no larvae have been recovered from the liver, lungs and intestines of newborn kittens after experimental prenatal infection. Martin (1926) attempted without success to produce prenatal infection of pigs with *A. suum,* stating that prenatal infection rarely, if ever, occurs naturally with this parasite. Matousek (1937) found no evidence of prenatal infection with *P. equorum.* These findings are in accordance with the tracheal migration exhibited by the last two species.

c. abdominal migration.

A third modification in migratory behavior is exhibited by the larvae of *Toxascaris leonina.* Fülleborn (1922) produced experimental infection of dogs with this parasite by feeding embryonated eggs. There was no evidence of somatic migration, most of the larvae being recovered from the abdominal viscera. Wright (1935) confirmed these findings and showed that development proceeded in the intestinal wall, most of the larvae returning without migration to the lumen of the intestine. The writer (unpublished) has studied the development of *T. leonina* and *T. mystax* in experimentally infected cats. It was found that in both species, development from the egg to the adult stage proceeded in the alimentary tract, no somatic infection being evidently necessary. Infection with both species could also be produced by feeding mice harboring the larvae in their tissues.

Life history patterns among the ascaridids.

Mozgovoi (1950) recognised the following life history patterns among the parasites in the suborder "Ascaridata": (1) migration through the liver and lungs as shown by the species in terrestrial mammals; (2) infection without migration of larvae as shown by the species in terrestrial birds; (3) prenatal infection, as shown by *Toxocara canis;* (4) infection through an intermediate host as shown by species occurring in aquatic animals. A revised and tentative scheme for life history patterns at present known in the Family Ascarididae is shown below:

1. Larva, after hatching, ingested by intermediate host, infection by ingestion of intermediate host.
   e.g. *Contracaecum spiculigerum, Raphidascaris acus, R. gigi* (Mozgovoi, 1953)
2. Embryonated egg ingested by intermediate host.
   a. Larvae remain in tissues of intermediate host until it is eaten by second intermediate or final host.
      i. Larvae in invertebrates,
         e.g. *Raphidascaris canadensis* (Insect larvae)

Contracaecum aduncum, C. clavatum, C. osculatum (Crustacea)
Goezia spinulosa (Crustacea), *Porrocaecum crassum* (earthworms)

ii. Larvae in fish,
e.g. *Raphidascaris canadensis, R. acus*
Contracaecum aduncum, C. microcephalum, C. osculatum
Porrocaecum decipiens? P. reticulatum

iii. Larvae in amphibia,
e.g. *Contracaecum spiculigerum*
Ophidascaris labiatopapillosa
Multicaecum tenuicolle

iv. Larvae in mammals, (rodents and insectivora),
e.g. *Ascaris columnaris*
A. devosi, Toxocara canis
T. mystax, Toxascaris leonina
Ophidascaris filaria
Porrocaecum depressum?

b. Migration of larvae to intestine and dissemination of infective larvae in feces (only a possibility),
e.g. *Ascaris suum?, A. lumbricoides?*

3. Embryonated egg ingested by final host.
a. Migration of larvae through liver and lungs to intestine,
e.g. *Ascaris lumbricoides, A. suum, A. tarbagan, Parascaris equorum, Toxocara canis* (in new born)
b. Migration of larvae through liver and lungs to somatic tissues, infection prenatal,
e.g. *Toxocara canis, Neoascaris vitulorum*
c. Migration of larvae into intestinal wall, larvae return directly to intestine,
e.g. *Toxascaris leonina, Toxocara mystax*

SUMMARY

Evidence for the utilization of intermediate hosts by members of the Family Ascarididae Blanchard, 1896 is summarized; the conclusion is reached that this is a widespread feature, occurring in both terrestrial and aquatic members of the two subfamilies Anisakinae and Ascaridinae.

The use of intermediate hosts is regarded as a primitive characteristic. The various species of animals at present known to be used for this purpose indicate the possibility that this family originated as parasites of marine arthropods, and that radiation has occurred amongst marine animals through the use of marine invertebrates and fish as intermediate hosts. The spread to terrestrial animals may have occurred through terrestrial coprophagous animals, such as rodents, swallowing ascaridid eggs from the feces of littoral animals. By harboring encysted larvae in their tissues, rodents may have become intermediate hosts for the ascaridids of many terrestrial carnivorous animals.

In extending their range to non-carnivorous hosts, the ascaridids have modified the migratory behavior of the larvae so as to dispense with intermediate hosts, and

promote infection of the final host through the ingestion of the embryonated eggs. In some species, e.g. *T. canis,* the somatic type of migration has been retained and infection occurs through penetration of the larvae into the fetus; in other species, e.g. *A. suum,* the somatic migration has been dispensed with in favor of tracheal migration; in *T. leonina,* migration has been limited to the abdominal region so that the larvae may return to the intestine directly.

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